

A Medal-seeker's Guide

ALL that most scientists get for their hard work is a pay-packet and a vague feeling of satisfaction that one or two of nature's secrets have revealed themselves. Higher rewards, however, are available—medals and prizes. Since the cash is often not insignificant, we provide as a service to our readers a guide to landing some of Britain's plum rewards that they may have overlooked or thought inaccessible. There are no preliminary qualifications and in general the nationality of potential recipients is not taken into account in making awards. The selection of winners is entrusted to the Royal Society whose yearbook provides our information. As *Nature* goes to press, this year's winners are announced, so there are twelve months to make an impact before the next award. The most recent winners are not included in the statistics, which cover the last twenty years and may be helpful as a guide to prospects.

Copley Medal, 1731, silver-gilt. From legacy of Sir Godfrey Copley, Bt., F.R.S. (1653–1709). Annual. £100 added. An additional £1,000 comes from a Jaffé prize. Nobel laureates will recall that they are not eligible for Jaffé prizes, but for them £1,000 is available from 'another source'.

Given annually to the author of research published by the Society deemed 'deserving of that honour'. In the middle of the nineteenth century the medal was "recognized as the 'palm and laurel' of science" (*Nature*, 174, 1034; 1954) and thirty-two of fifty awards between 1851 and 1900 went to foreigners.

To two Foreign Members in the last twenty years, otherwise to Fellows of the Royal Society, generally of twenty years' standing.

Rumford Medal, 1800, silver-gilt. By Count Rumford, F.R.S. Biennial. £200 added.

Given to the author of the most important discovery in any part of Europe in Heat or Light.

To a Dutchman in 1964, otherwise to Fellows on each occasion in the last twenty years.

Royal Medals, 1825 and 1965, gold. Three available. Annual. No money.

Given for the most important contributions in pure and applied science published in Her Majesty's Dominions.

Of forty-eight recipients, forty-six were already Fellows. Helpful to have been one for at least ten years.

Davy Medal, 1877, bronze. Of obscure origin believed to be connected with pawning of Sir Humphry Davy's tableware. Annual. £200 added.

Given for the most important discovery in chemistry in Europe or Anglo-America.

Not much good chemistry outside Britain these days. Of last twenty recipients, one Swiss, two American and seventeen Fellows of the Royal Society.

Darwin Medal, 1890, silver. Biennial. £200 added.

Given for work of acknowledged distinction in the field in which Charles Darwin laboured.

No limitation of nationality or sex. One Foreign Member, nine Fellows in last ten awards. All male.

Buchanan Medal, 1897, silver-gilt. Quinquennial. £200 added.

Given for distinguished work in Hygienic Science or Practice.

No limitation of nationality or sex. Two of last four winners were not Fellows, but all were male.

Sylvester Medal, 1901, bronze. Triennial. £200 added.

For the encouragement of Mathematical Research, irrespective of nationality (sic).

Last six recipients should have needed no encouragement to carry on, as they had been Fellows for on average twenty years.

Hughes Medal, 1902, silver-gilt. Given by Professor David E. Hughes, F.R.S. Annual. £200 added.

Given for original research, particularly in Electricity and Magnetism.

No restriction on nationality or sex, but the last twenty recipients have all been male Fellows.

Leverhulme Medal, 1960, gold. To mark the tercentenary of the Society. Triennial. £500 added.

Given for the most significant contribution in chemistry or engineering.

In 1966, Sir Alec Issigonis won this medal before being made a Fellow.

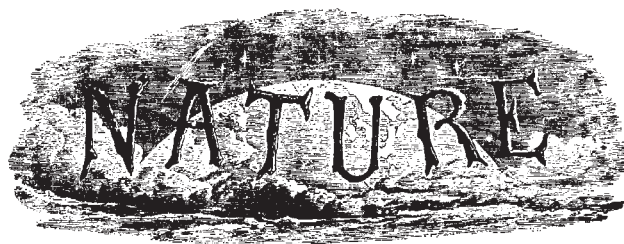
Mullard Medal, 1967, gold. Annual. £1,000 added.

The only medal for which Council formally takes outside advice.

Given for an outstanding contribution to science, engineering or technology leading directly to national prosperity in the United Kingdom.

Not awarded to a Fellow since 1967.

100 Years Ago



WE learn from the Journal of the Society of Arts, that one of the first results in the rise of the price of coal has been the formation of a company in France, whose object is to utilise the power of the ocean tides on the French coast by proper machinery. The first experiment is to be made at St. Malo, where the tide rises nearly 80 ft., and overflows many square miles of flats.

From *Nature*, 9, 54, November 20, 1873.



Analysis of Cause—Long Cut to Prevention?

"Abatement is the aim of medicine . . . even the concept of causation can, on occasions, be dispensed with in considering abatement strategies."

Dr J. H. Renwick of the London School of Hygiene and Tropical Medicine urges the need for more emphasis on preventative thinking in framing hypotheses in medical science.

ALTHOUGH in biomedical research prevention of a disease is customarily considered complementary to its cause, most of the scientific working hypotheses are framed around testing the supposed cause directly. In some circumstances, however, there are advantages in framing hypotheses around prevention.

I shall consider first the circumstances in which the classical causal hypothesis is more or less adequate. These arise either when a disease situation occurs naturally that superficially resembles a laboratory experiment or when a true experiment is possible in an animal that is acceptable as a valid analogue of man.

The thalidomide incident is an example of the natural or at least unplanned experiment. When thalidomide was taken by pregnant women and a defined range of otherwise rare malformations was observed in many of their offspring but not in the offspring of other women, the hypothesis was

proposed that thalidomide was at least a contributory cause of these malformations in humans. Indeed, for any new ailment (disease, malformation or injury), it is simple and effective to state a proposition in the causal mode. As in a classical experiment, a known background situation is modified and an effect produced.

An animal experiment with thalidomide would be an example of the other situation (of adequacy of a causal hypothesis) but only when the test animal is trusted as a valid analogue of man. Rhesus monkeys and certain marmosets are so trusted; the malformations produced in them by this drug are almost identical with those in man. A causal hypothesis even for man, being therefore indirectly testable, can usefully be proposed.

Need for Abative Hypotheses

The position is different for ailments to which man has been subject for a long time and for which no convincing animal analogue is available. Unfortunately, we are in the habit of constructing causal claims about even these ailments although the relevant experiments to test the claims directly would usually be unethical. The causal experiments are therefore impracticable except in imagination. In a 'thought experiment' of this sort, an artificial base-line for the population has to be created. In one example, it could involve the (imaginary) absence of refined sucrose. This basal situation would then be modified (in imagination) by the addition of sucrose and various alimentary disorders would ensue. Such at least is the experiment that seems to be suggested by a causal claim that sucrose is an important factor in the cause of colonic diverticulosis, for example. The hypothesis cannot be tested directly because it is nearly always unethical to increase the risk of ailment in a real experiment.

If we can overcome the habit of allowing cause to dominate our thinking, we have a very simple way of bypassing such a problem. This is to perform a real intervention experiment on a part of the population with its present, known burden of disease. This basal situation is then modified—by some procedure such as reduction of intake of refined sucrose. The degree of abate-

ment of the disease is then the outcome of the experiment. With such an experiment in mind, a preventative hypothesis can be formed that is in principle testable, and there are usually no major ethical objections to an experiment of this sort. Further, the statement can be framed briefly yet accurately in terms of deviation from the present state because the 'present state' is known widely enough for it to require, in many cases, no detailed description. Many intervention experiments of exactly this type are done. The change that I am suggesting is that they should be acknowledged as testing preventative statements and not causal ones.

Preventative statements, when confirmed, will sometimes summon up practical measures to control disease. Any such measure—it may be preventative or therapeutic or both—will be called 'abative' as its purpose is to produce some abatement of the ailment and of its burden to the individual and to society.

Non-reciprocal Relationship between Cause and Abative

Wherever a necessary-and-sufficient abative measure exists there must, in principle, be a corresponding necessary-and-sufficient cause and *vice versa*. The search for cause or abative then comes to much the same task. But the relationship between cause and abative is not always so exactly reciprocal. For instance, a sufficient abative is one that removes a necessary cause. A somewhat oversimplified example of a 'sufficient abative' would be the banning of rye cultivation to prevent poisoning by the ergot fungus, for which rye is the preferred host. The corresponding causal statement is that rye is the 'necessary cause' of ergot poisoning. As in this example, so in general—an abative measure that has the property of sufficiency but not of necessity is the counterpart of a cause that is necessary but not sufficient.

The degree of reciprocity is also limited in a more subjective way. Even though abatatives, like causes, do interact, the percentage abatement of a disease incidence by a single manipulation is an unambiguous and complete concept. By contrast, the concept of the percentage effect of a single cause is more likely to be misleading unless the inter-

actions with other causes are fully specified. Specifying these interactions by the disclaimer—other things being equal—is rarely adequate.

Few such difficulties apply to statements or hypotheses on abatement. The effects of single causes or abatives are best expressed as percentage change from the initial situation. The effects (causal or abative) may sum to several times the incidence of the disease. This is not awkward if it is abative effects that are being summed since each is operative only potentially and mainly in the future. We are not surprised at the existence of alternative means of prevention nor at the possibility of their interacting, so when the sum exceeds 100% effectiveness, we are not disturbed. But there is a seeming absurdity in the statement, say, that 50% of the present incidence of a disease is caused by each of three factors, x , y , z . For the claim to be explicit, interactions or options must be invoked because, obviously, the realised effect of the three factors combined does not exceed the present overall incidence.

As already noted, intervention studies of abative measures are already an established part of epidemiological practice. For example, there have been non-smoking studies, breathalyser studies, and, on the positive side, fluoridation studies. But the conceptual framework has not been adequately spelled out to my knowledge. In some intervention studies, by a curious inversion, the investigators even imply that causes are being studied rather than abatives. The distinction is worth making as it reflects a difference. Abatement is the aim of medicine; the study of causation of an ailment, say, in experimental animals, is frequently a useful intermediate but may not be an essential one in all cases. Even the very concept of causation can, on occasion, be dispensed with in considering abatement strategies.

Remaining Problem Diseases are a Selected Sample

I suggest that if neither causes nor abatives are yet known—and I shall call such an ailment a problem ailment—causation is probably complex. The more complex the causation, the more likely it is to be currently unknown. In tackling such a problem ailment, then, we would be wise to turn at least some of our attention from causes to abatives. For example, the causal mechanism of carcinoma of the colon is unknown and probably complex. From its geographical distribution the simple and testable abative measure of a high residue diet has been proposed. From past neglect, simple abatives like this

have not always been adequately excluded.

Advantages of Abative Statements

For old-established conditions, three major advantages of abative compared with causal statements have so far been mentioned—direct testability on the human species itself; expressibility in simpler terms; and the opportunity to include a quantitative component that expresses the percentage of prevention anticipated.

This last advantage is well illustrated in the case of road accidents. We should like to make statements such as “20% of accidents in the United Kingdom are caused by alcohol”, but such statements are intrinsically misleading because of the interactions: practically no cause operates in isolation. An abative statement that the introduction of the breathalyser laws in the United Kingdom reduced the accident rate by a certain percentage is simpler and open to a smaller range of interpretations.

This example introduces another important advantage of abative propositions. They are directly relevant to the achievement of human goals such as the partial prevention of accidents, and so are directly relevant to decision making in public and private life. The quantitative aspect is the most important in this context, as the predicted advantage has to be balanced against the cost of introducing the abative measure.

It is at least arguable that if the observed association between smoking and lung cancer had led to an abative rather than a causal claim, society's 20 yr delay in taking action would have been shorter. The claim would have been directly confirmed by the results now available of non-smoking intervention studies and the question of the identification of the presumed carcinogen(s) could have been deferred. Further, the genetic red herring would have been more clearly recognised for what it is—a matter of intellectual interest but with little practical relevance, in this instance, in decision making.

Perspectives on Causation and Abatement

The properties of abative statements are so attractive that we need to ask why they are rarely made. After all, if prevention of disease is an aim of medicine, why have we departed so completely from the folklore mode of expression exemplified by ‘an apple a day keeps the doctor away’? The historian might well find an influence of the philosophy of non-medical science—

the dominance of how (does it happen) over how not. Waddington in *The Scientific Attitude* roughly defined science as “the organised attempt of mankind to discover how things work as causal systems”. Perhaps the absence of specific mention of abatement in such definitions has been taken to imply that abative statements are less scientific than causal ones, despite the fact that, for both types, the “criterion of truth is experimental” and objective.

Alternatively, did the current emphasis on causation spring from the need of the legal system to apportion blame? Or, equally plausibly, was the need to study epidemics more pressing than the need to study the less dramatic non-epidemic state? The manifest success of laboratory methodology in so many non-human fields undoubtedly played a large part in establishing the habit of thinking in the causal mode. We correctly believe that we have not understood anything at a fundamental level unless we have understood the mechanism of causation. And we often think (incorrectly) that such understanding is a prerequisite of wise action. Kant knew better—that “it is often necessary to make a decision on the basis of knowledge sufficient for action but insufficient to satisfy the intellect”.

It is true, even today, that wise action can occasionally be based at least temporarily on empirical knowledge alone and there are many examples of this from the past. From the time of Admiral Wager, scurvy was controlled in the Navy by the provision of citrus fruits before ascorbic acid was known to be a vitamin. Penicillin was used effectively, even before its mode of action was understood; and tobacco avoidance dramatically reduces bronchocarcinoma incidences although we still do not know how.

Decision-making in medicine is more directly concerned with prevention and treatment rather than causation, so we should often express claims in terms of abatement rather than causation as at present. A few scientists disagree. They argue that causation and abatement are simply converse concepts and, at the same time, that one is scientific, the other empirical. Both arguments are rather shallow assessments. Abatement is not always the simple converse of causation. Further, an abative statement can be as scientific and testable experimentally as any other. It may even be just as possible to discover general laws concerning abatement as laws concerning causation.

Abative and causal hypotheses are not mutually exclusive. Indeed, there is a one-to-one relationship between them. The abative statement is usually simpler in practice. This is particularly true for complex problems. And most real-life problems are complex.

NEWS

Slower and Cooler in the United States

by our Washington Correspondent

IN the next few months, nearly every American is going to be brought face to face with the energy crisis, for it is now clear that there may be rationing of petrol and heating oil, closing of some schools and offices, shorter working hours and even a few cold homes. Apart from the inconvenience and upset that these situations will cause, their overall effect will be to force Americans to cut back on their use of energy—not just this winter, but permanently. And that will require some rather fundamental changes in a society that has been mindlessly consuming energy at a staggering rate.

Last week, President Nixon went on television to warn the country of the gravity of energy shortages that are likely to develop in the United States this winter, and to outline emergency measures that his Administration wants to take to deal with the situation. The reaction has been startling. Newspapers have been filled with little else—for a time the energy crisis even eclipsed Watergate as the focus of national attention—state governors have been announcing measures to curtail consumption of energy in their territories, and a few people have turned down their central heating. On Capitol Hill, committees have been stung into action and have passed legislation to give the President sweeping powers to force a reduction in the use of energy throughout the country. And the Stock Exchange has seen the biggest drop in share prices in 11 years.

The root cause of the impending shortages is that the growth in energy consumption in the United States has for years been outstripping the growth in supply. But the situation has been blown into crisis proportions by the embargo that Arab countries have placed on their oil exports to the United States, an action which will strip 2 million barrels of oil a day off the supply. The Arab cutoff, together with shortages that were developing in any case, could result in oil supplies falling short of demand by 17% when winter finally arrives. The problem, however, is that the shortages will not be evenly distributed, for it is predicted that the gap could reach an incredible 40% in the Northeast, an area which relies heavily on imported oil.

That is the prospect which caused President Nixon last week to deliver his third energy message to the American public so far this year. Unlike the pre-

vious messages, which have been concerned chiefly with increasing energy supply, and which have paid only scant lip service to the need for reducing demand, the latest effort was concerned almost exclusively with emergency measures that must be taken to decrease consumption of energy. The message came too late, and it was only forced by what President Nixon referred to as "the most acute shortages of energy since World War II", but nevertheless it represents a fundamental break with the Administration's previous attempts to deal with the energy crisis.

What Nixon is asking, in short, is for Congress to give him broad emergency powers to force people to curtail their energy appetites, and he is also appealing to the American public to act voluntarily.

Among the measures outlined in his message are the following:

- The Administration wants the power to order neon lights and ornamental lighting to be turned off, a measure which would have the welcome effect of getting rid of some of the neon nightmares that encircle so many American cities.

- President Nixon is requesting authority to force shops to close early, to require speed limits to be lowered on all roads, and to impose emergency taxes on consumption of natural gas and on excessive uses of electricity.

- He wants Congress to pass a bill authorising the use of daylight savings time (the equivalent of British Summer Time) throughout the year—a measure which could save up to 3% in electricity consumption, particularly in the northern areas which are most affected by lack of fuel.

- Finally, he is asking for broad authority to allow power plants to be exempt from air pollution regulations, so that they can burn coal and oil containing relatively large quantities of sulphur.

Those measures all require Congressional approval, but the Administration already has powers to force other changes, and it is using them. Regulations are being drawn up to prevent factories and power stations from switching from coal to oil, and the Administration is also putting pressure on electricity producers to convert power plants from oil to coal. The White House says that 46 plants can be converted within two months, at a saving of nearly half a million barrels of oil a day. The number of commercial jet flights in the United States has been reduced by the

Federal Aviation Administration, and Nixon decreed that thermostats be turned down in federal buildings. He also appealed for central heating to be turned down in private homes, for cars to be driven at speeds less than 50 m.p.h., for more use of mass transit, and for more car pooling.

Scarcely were the words of exhortation out of President Nixon's mouth when Congress was galvanised into unprecedented action. Senator Henry M. Jackson, chairman of the Senate Interior Committee and the most influential member of the Senate on energy matters, rammed a bill through his committee, after a day of hearings in a cool committee room, and the House Commerce Committee also expects to get a bill passed this week. Thus Congress is likely, by the end of next week, to have given President Nixon most of the powers he requested.

The emergency measures proposed by Nixon seem tougher, at least if implemented, than the British government's proposals announced on Tuesday in the face of a crisis exacerbated by a miners' overtime ban.

But will they be sufficient to cope with the situation? By all accounts they will not, and the Administration is developing plans to ration petrol and fuel oil if the need arises. For one thing, the measures announced last week will rely heavily on public cooperation and voluntary compliance, and for another, some of them have come too late to be effective. For example, the proposal to convert power plants from oil to coal—a fuel which the United States has in abundance—is likely to be stymied by the fact that coal production is already taking place at maximum capacity, and thus it is unlikely that coal output can be increased in time to meet a new surge in demand. Moreover, according to the White House's most optimistic forecasts, the proposals will cut demand for oil by some 2.3 million barrels a day, a figure which is well short of the predicted gap between supply and demand of three million barrels.

Thus, senior Administration officials are warning that it is likely that oil and gasoline rationing will have to be brought in before the winter has ended. Moreover, the problems will not disappear at least until the end of the 1970s when the Alaska pipeline will be bringing crude oil to the lower states. This winter is therefore likely to mark the end of an era of cheap, bountiful energy in the United States.

ENVIRONMENT

Environmental Protection

by our Washington Correspondent

THE House Appropriations Committee has handed both the Environmental Protection Agency and the National Academy of Sciences a political hot potato which could prove embarrassing. The committee passed a bill which includes \$5 million for the EPA to let a contract with the academy to carry out a massive study of the EPA's programmes and past decisions. That provision was eventually passed by the House of Representatives and the Senate, and it was signed into law at the end of last month. If the academy agrees to accept the contract, it will undertake one of the biggest and most difficult studies it has ever been involved with, and it will also run the risk of being used by hostile Congressmen as a weapon in their campaign to emasculate the EPA.

The appropriations bill itself carries the bare statement that \$5 million be provided for the academy to study the EPA, but the House appropriations committee report spells out the nature of the study in some detail. The committee suggests that the study should involve an estimate of the costs of pollution abatement over the next 10 years and that it should include an analysis of the costs and benefits of the pollution control programme—an analysis which should bring in social and economic factors.

The committee also propose that the academy be given the task of examining the costs and benefits of automobile emissions controls, the extent to which environmental regulations have contributed to the energy crisis, and the effect on agricultural productivity of various decisions to ban pesticides. Virtually every one of those topics has already been the subject of intensive study and heated debate, and there are many who find it difficult to see what new insight the academy might be able to bring to the matter.

Why was the provision put into the bill? The answer, in short, is that the EPA is unfortunate enough to have its budget scrutinised by the agriculture subcommittee of the House Appropriations Committee, and many powerful members of the subcommittee, being closely allied with farming interests, regard the EPA with about as much affection as they do the boll weevil. The chairman of the subcommittee, Jamie Whitten, a conservative Democrat from Mississippi, makes no attempt to conceal his contempt for the EPA. Last week he told the House of Representatives that the agency has contribu-

ted to the energy crisis, increased the costs of food production, increased the danger to human health by banning DDT, and put American agriculture at a competitive disadvantage.

Thus the committee is looking to the academy to provide intellectual support for its complaints about the EPA, which is an unenviable position for the academy and for the EPA to be placed in.

SOUTH EAST ASIA

North Vietnam Appeal

by our Washington Correspondent

THE Scientists' Institute for Public Information (SIPI), a public interest organisation, has launched an appeal in the scientific community for help in repairing some of the destruction caused by US bombs, herbicides, shells and other weapons in Southeast Asia. The immediate goal is to establish a Research Institute for Agricultural Botany in North Vietnam—a project which has been specifically requested by North Vietnamese scientists to help revegetate rural areas which are extensively littered with bomb craters.

According to Dr Barry Commoner, past president of SIPI, the appeal will serve the dual purpose of alerting US scientists to the post-war situation in North Vietnam and of informing the US public of the need to provide aid to all of Indochina. This is a promise which in fact was made by President Nixon during the cease-fire negotiations but which has since fallen into political disfavour.

The idea for the agricultural research institute stems from a visit to North Vietnam made in July this year by two American scientists with the support of SIPI. Dr E. W. Pfeiffer, a zoologist from the University of Montana, and Dr Arthur Westing, a botanist from Windham College, Vermont, returned with reports of devastation on a scale which Dr Westing said last week was "almost unbelievable". He said that they were rarely out of sight of bomb craters, even in the most rural areas of North Vietnam, and since more than 90% of the population is directly engaged in agriculture, Dr Westing pointed out that the greatest need for aid lies in rehabilitating the rural areas.

The SIPI appeal started this week with a letter sent by Dr Commoner and Dr Arthur Galston, professor of botany at Yale University, to some 10,000 scientists asking for aid in establishing the research institute, for scientific books and journals to be sent to North Vietnamese scientists and for equipment for the institute. Dr Galston said last week that \$100,000 will be enough to start the institute off, and that SIPI is hoping to get some of the money from

institutions and foundations. He reckons that sufficient cash and equipment should be available by April 1974 to send a small team of US scientists to North Vietnam to work in the research institute. In addition, SIPI has arranged to ship scientific books and journals to South Vietnam by way of Dr Philip Harvey, of St Stephen's Hospital in London.

OTA

Daddario Appointed

MR EMILIO Q. DADDARIO has, as expected, been appointed the first director of Congress's new Office of Technology Assessment. The appointment puts Daddario in charge of a body which he is largely responsible for setting up, and brings him back to Capitol Hill three years after he left the House of Representatives to make an unsuccessful bid for the governorship of Connecticut.

A lawyer by training, Daddario was elected to Congress in 1958, and in 1963 he became the first chairman of the subcommittee on Science, Research and Development. Under his stewardship, the subcommittee rapidly established itself as the focal point for discussion of science and technology policy issues in Congress. It also rather grotesquely revamped the National Science Foundation by making its top officials direct Presidential appointments and enabled the NSF to sponsor applied research. The subcommittee began hearings on research into environmental problems before the environmental bandwagon had even begun to move, and drew up the legislation which eventually led to the establishment of the Office of Technology Assessment.

Daddario's appointment was confirmed when the OTA board met early in November—its first meeting since the office finally got some funds to begin work (see *Nature*, 246, 6; 1973). Next step is to appoint a deputy director and an advisory council. The most likely candidate for deputy director is Daniel V. Simone, a former member of the defunct Office of Science and Technology, who is now on the staff of the NSF's Office of Science and Technology Policy. There should be little problem in finding good staff for the OTA—some 3,500 people have applied for jobs there, even though none has been formally advertised.

Short Notes

CSII on Ice

THE Centre for the Study of Industrial Innovation is in danger of dying from neglect. Negotiations between the centre's directors and the Cranfield Institute of Technology, which it was hoped would lead to the centre becoming a department of industrial innovation at the institute, have scarcely moved in the past nine months.

A recent meeting between Mr George Teeling-Smith, the centre's director, and Dr Henry Chilver, the Vice-Chancellor of Cranfield, means that discussions are still continuing, but the centre has effectively been out of operation since January of this year.

Mr Teeling-Smith said this week that it is "disappointing that it is taking so long" for any changes to take place. "We have lost so much impetus that effectively we will be starting from scratch again." At present CSII has nothing to do except sell back copies of its reports on industrial innovation, which include an assessment of the spin-off benefits from Concorde, an appraisal of the industrial research associations and the publication of the results of the Project Sappho studies on industrial innovation undertaken at the University of Sussex.

Dumping at Sea

A BILL to control dumping at sea has just been introduced to parliament. To date dumping has been limited by voluntary agreements between industry and government. Over the past seven years this has been quite effective but the bill will formalise the voluntary arrangement, making it necessary to acquire a licence to dump any material in the sea, and introducing heavy penalties for offences.

If the bill is passed Britain could be the seventh country to ratify its signature to the Oslo convention agreed in February 1972, which dealt with dumping in the north-eastern Atlantic. This convention requires ratification by seven countries to come into force. The bill will also enable Britain to ratify the London convention, concluded in December 1972, which was a global convention on similar lines to the Oslo convention. This requires ratification by fifteen countries to come into force, and Britain will probably be only the third country to ratify it. It is not, however, as immediately important to British fishing as the Oslo convention, which is concerned with the sea around the British Isles.

The basic determinant on which the decision to grant a licence will be based is the effect of the dumped material on fish. Certain substances which persist in the environment are blacklisted,

and licences for their dumping will never be granted. These include mercury, cadmium, and DDT. For dumping of other materials, including arsenic, lead and fluorides, a special licence is required, and the materials must be dumped in deep water, at least 150 nautical miles from land. Factors such as the concentration and form of the material and the area in which it is to be dumped will be taken into account in the issuing of all licences.

Dumping at sea is responsible for only 5-10% of shore-generated marine pollution. The main sources, streams and pipelines, are not affected by this bill, but will be dealt with in another bill to be published shortly, concerned with many forms of pollution including atmospheric and noise pollution. Neither does the present bill cover atomic waste, which was dealt with by the act of 1969, nor with pollution from ships, which was discussed last month in a convention in London.

Solar Energy in Australia

AUSTRALIA is expanding its interest in solar energy business with some enthusiasm. Last week a Solar Energy Studies Unit was formed by CSIRO under the direction of Mr R. N. Morse, formerly chief of the CSIRO Division of Mechanical Engineering, and immediate past president of the International Solar Energy Society.

CSIRO has had an interest in solar energy for almost twenty years, and solar heating of water is already in use to the extent of saving Australia 5,000 tonnes of coal a year. Solar heated air is also used in some industrial drying processes, while experimental timber drying kilns are already being tested.

The new unit will look at the most promising applications of solar energy for Australian conditions. Feasibility studies of short and long term projects will be undertaken, focusing current CSIRO fundamental work on solar energy collections, heat storage media, energy measurement and thermal transfer problems.

Mr W. L. Morrison, Australia's minister for science, announcing the appointment said that "the use of solar energy on a wide scale for domestic and industrial heating could help substantially to offset increased costs of fossil fuels in the future".

Developing Research

THE National Research Development Corporation is alive, well and even flourishing. This is the message contained in the corporation's annual report published last week (available free from NRDC, 66 Victoria Street, London, SW1).

The NRDC has come in for some criticism recently, particularly at the

hands of the Select Committee on Science and Technology which recommended that the corporation's structure needed "urgent financial review". This criticism was included in the select committee's report (published in September) on the closure of Tracked Hovercraft Limited, an NRDC subsidiary.

Dr Basil Bard, the corporation's managing director, said last week that the forecast for the future is encouraging with a net surplus for 1972-73 reported of £529,000 compared with £178,786 the previous year. The total income of the corporation went up from £7.4 million to £8.7 million.

One of the problems which the corporation is faced with, according to the report, is that of "maintaining in industry, in the universities and in other sources of invention, a knowledge of the corporation's existence and of the facilities it is able to provide for promoting innovation in industry".

Dr Bard said that in the past year uncertainty about the corporation's future had been replaced "by an increased awareness of the need for a body like the corporation, which has encouraged us in our belief that we have an important part to play in providing financial aid for the development and commercialisation of significant UK innovation". This, however, does not take any heed of the select committee's views.

Comet Kohoutek Disaster?

DR D. W. DEWHIRST of the University of Cambridge warned a meeting of the Royal Astronomical Society last week that members should be cautious in their public statements about Comet Kohoutek. Little is known about the factors that govern a comet's brightness, he said, and the prediction that Kohoutek will be a bright object in daylight may be over-optimistic. According to his calculations the brightest that the comet will be as viewed from Britain is magnitude -3 (on January 3) which is about as bright as Venus. Perhaps Dr Dewhirst has recalled that the failure of the Leonid meteor shower to live up to expectations in 1899 was described by the meteor scientist C. P. Olivier as "the worst blow ever suffered by astronomy in the eyes of the public".

More Mobility

SIR HERMAN BONDI's task force to encourage the mobility of scientists is nearing the end of its appointed task. Its report is being written at present and will be submitted to the Lord Privy Seal at the end of the year or in January.

There is no news of what the report contains but it is highly unlikely that the work started by the task force will be allowed to come to an end with the publication of the report in the Spring.

NEWS AND VIEWS

Changing View of Operator-Repressor Interactions

ONE of the most critical steps in the development of theories of gene control was the concept that some sequences of DNA may function not by their transcription into RNA and subsequent translation into protein sequences, but rather by serving as signals which are recognised by protein molecules acting as regulators or enzymes of gene expression. Elucidation of the mechanism of such protein-DNA interactions has become a principal aim of current research, in particular concerning the promoter sequences recognised by RNA polymerase and the operator sequences recognised by repressor proteins. The two systems which have proved particularly productive, the lactose operon of *Escherichia coli* and the early development of phage lambda, now seem likely to present very different pictures of repressor-operator interactions.

Operators have in general been thought of as rather short sequences of DNA; a consensus of opinion which is presently evolving is that they comprise, or at least include, symmetrical sequences of base pairs. A sequence of the order of twenty base pairs with features of symmetry seems, for example, to constitute the operator of the lactose operon. But the most recent work of Maniatis and Ptashne, reported on page 133 of this issue of *Nature*, replaces the idea of a single short sequence with the concept that in lambda the two operators may each comprise more complex structures, capable of recognising several repressor molecules.

The two lambda operators comprise sequences of DNA to which the lambda repressor protein binds to maintain the phage in an inactive state, by preventing transcription. Repressor binding to the 'left' operator prevents synthesis of the RNA corresponding to gene *N* immediately to the left of O_L and repressor binding to the 'right' operator similarly prevents transcription of gene *tof* lying immediately to the right of O_R . In the experiments which they now report, Maniatis and Ptashne take advantage of the techniques reported earlier this year by Pirrotta (*Nature new Biol.*, **244**, 13; 1973) for isolating the lambda operators. Radioactively-labelled lambda DNA, sonicated to yield fragments of molecular weight $3-6 \times 10^5$, is bound to the lambda repressor protein and, in several subsequent steps, the sequences of DNA not protected by the repressor are degraded with DNase and the operator sequences are retrieved by binding the repressor-operator complex to nitrocellulose filters. Since the yield of protected DNA is twice as great with wild type lambda DNA as with a mutant possessing only one operator, both of the operators of lambda can be isolated in this system.

In early experiments, the fragment of DNA protected by repressor corresponded to some twenty base pairs in length. This is much larger than had been anticipated, because in its active dimer form the repressor protein should cover only some ten to fifteen base pairs of DNA. That the additional length results from the ability of each operator to bind more than one repressor was shown by Maniatis and Ptashne earlier this year (*Proc. natn. Acad. Sci. U.S.A.*, **70**, 1531; 1973), when they found that upon digesting lambda DNA with nuclease in the presence of

lambda repressor, the size of the protected fragments increases as the ratio of repressor to operator increases. The smallest fragment corresponds to a length of some thirty-five base pairs, the size then increasing with the repressor/operator ratio to 45, 65, 75, 85, 100 base pairs. By comparing the pyrimidine tract sequences obtained from the left operator, they were able to show that each increase in size of the protected fragment represents the addition of a short sequence to the fragment immediately preceding in the series. This suggests a model in which the operator consists of a sequence about thirty base pairs long which binds a dimer of repressor; following binding to this primary site, additional monomers of repressor may add to sequences about fifteen base pairs long adjacent to the primary sequence. A further indication of the complexity of the operator-repressor interaction in lambda is provided by the observation that, although displaying similar hierarchical structures, the left and right operators constitute different sequences—they generate different pyrimidine tracts.

Attempting to define more precisely the structure of the lambda operator, Maniatis and Ptashne have now (page 133) turned to an ingenious exploitation of two restriction endonuclease enzymes which introduce double strand breaks at different sites in the lambda molecule. When lambda DNA is digested with the Hpa enzyme derived from *Haemophilus parainfluenzae*, it is cut in such a way that two fragments are released which contain the operators; a segment of 350 base pairs contains the left operator and a sequence of 2,000 base pairs includes the right operator. Using the second endonuclease, the Hin enzyme of *Haemophilus influenzae*, each of these fragments can be cut into two subfragments. All four subfragments can bind repressor protein, suggesting that each intact operator can be divided into at least two sequences each of which can independently bind repressor *in vitro* in these conditions.

The order of the repressor binding sites within the operator was assigned by taking each of the fragments of length 35–100 base pairs, previously isolated by degrading the repressor-operator complexes formed with different ratios of the two components, and following the effect of the Hin endonuclease. Each fragment was split into two by the Hin enzyme, one subfragment always constituting a length of thirty base pairs, the other corresponding to the remaining length of the particular O_L fragment. What this means is that the primary repressor binding site (that is the fragment of thirty-five base pairs) lies at one end of the operator sequence, the secondary monomer binding sites extending from it in one direction only, comprising about four units of fifteen base pairs each. The action of the Hin enzyme is therefore to separate the primary binding site from whatever secondary sites are attached to it. Similar results are obtained when the right operator fragments are used instead of the left operator—either can be obtained by using mutants of lambda in which one operator lacks affinity for repressor—so that both left and right operators show the same general form of organisation.

By splitting intact lambda DNA with the *Hin* enzyme, Maniatis and Ptashne orientated each operator sequence. They found that the primary binding sequences of thirty base pairs of the left operator can be retrieved in the same fragment as a sequence corresponding to part of the *N* gene; similarly, the corresponding site for the right operator can be reclaimed in a sequence also containing part of the *toF* gene. The primary binding site therefore lies immediately adjacent, or at least very close, to the genes whose expression is prevented by operator-repressor binding; the secondary sites then extend away from the structural genes which are controlled.

Although these results provide a firm basis for some concepts previously held on less sure grounds, they raise several intriguing questions about the precise nature of the lambda operator-repressor interaction. Most notable, of course, is the dramatic difference between the short sequence of the lactose operator and the complex structure in lambda. An important observation, supporting previous speculations, is that the two lambda operators possess different sequences, and as a result have different affinities for the repressor protein, but nevertheless both function to prevent transcription. Also significant is the difference in affinities for repressor of the various sequences included in each operator. Whatever the nature of the DNA sequences which are recognised by the repressor, they can therefore vary in a way which may in future experiments come to throw an important light on the manner of protein-nucleic acid recognition. A puzzling point is the dependence of the secondary sites upon the primary site in the intact lambda DNA molecule; point mutations, apparently located in the primary binding sites, can prevent repression and so allow development of the phage. This means that inactivation of the primary site renders the secondary sites also unable to bind repressor in such a way as to repress transcription.

A final point of speculative interest is the location of the sites on which the restriction enzymes act. The restriction enzymes, of course, are part of the host cell modification and restriction system, which methylates certain sites of DNA native to the cell and also degrades any DNA lacking the appropriate methylations. It is interesting that the sequences recognised by these enzymes should lie so usefully in the control sites of the phage. One possible reason is that restriction and modification activities rely on symmetrical sequences of DNA, as may also the repressor recognition of DNA, providing an obvious resemblance between the two types of sequence. But in teleological terms, it is possible to wonder whether it is only such a coincidence that locates the host modification and restriction sites precisely where they can do most damage to development of the DNA if endonuclease action occurs.

From our Molecular Genetics Correspondent

Defence Mechanisms in Fish

THE capacity of fish to produce antibody on induction with antigen has been known since the early 1900s, and that this production is temperature dependent has been evident since the 1930s. The range of temperature dependence is related to the fish's natural environment, for example, in the warm water carp (*Cyprinus carpio*), antibody can be induced at 20–25° C but not at 12° C, whereas the sablefish (*Anaplopoma fimbria*), from cold waters

in the North Pacific, will produce antibody at 5–8° C. The induction phase of antibody production in fish is prolonged in relation to mammals—18–30 days for cold and temperate species—though some warm water species will produce circulating antibody in less than 9 days. The antibody titres in fish, however, will often equal or exceed the titres obtained in mammals and the circulating antibody can persist for much greater lengths of time—up to one year.

It might be argued that a low temperature which prolongs the induction of antibody will also reduce the virulence of a pathogen, simply by decreasing its metabolic activity. Accordingly, work has been directed to rendering cultured fish immune to various diseases by using the animal's specific immune mechanisms. This work, although still experimental, has met with considerable success though large scale trials on fish farms have generally not been so satisfactory. Nevertheless the fact remains that many fish seem to be severely handicapped and killed by various diseases in a much shorter time than that required for them to produce a measurable immune response, even when they are held in the most favourable conditions yet made available in laboratories. Although some valuable work is being carried out to investigate methods of immunising cultured fish populations (oral vaccination is of necessity the choice because of the large numbers of fish involved), much more ground work is required before applied research can be meaningful; for instance, little is known of the portal by which many important pathogens gain entry into the host fish. It is already known that antibody can be present in localised concentrations according to the site of application of the immunogen, for example, if antigen is administered intravenously the antibody will subsequently be located in the serum, whereas if antigen is fed to the fish in the food, antibody will subsequently be located in the gut mucus. Thus, for protection it would be important that antibody be induced at the site where the pathogen normally enters the host.

Apart from phagocytosis, the non-specific defence mechanisms have not been intensively studied in the lower vertebrates. In this respect the reticuloendothelial system is extensive in the spleen, kidney, heart, liver and gills of many fish species studied. More recent work, however, is providing evidence for the presence of non-specific substances with possible potent protective activity in the tissues and sera of lower vertebrates. The presence of some of these substances seems to be induced, whereas others may be present as normal constituents; for example, the production of an interferon-like substance in fish is elicited by virus and has been shown to confer considerable protection on susceptible cells. Serum titres of lysozyme have been demonstrated to increase (some 100 times) with increase in temperature and immunisation with bacteria, the increase in lysozyme titre being somewhat in advance of antibody production. This finding is contrary to what has been observed in homeotherms. C-reactive protein (CRP) appears in the serum of mammals during injections with microorganisms and has a non-specific precipitating activity. It is now shown by Baldo (see page 145 of this issue of *Nature*) that a substance which seems to have identical activity to human CRP is present in fish serum and will precipitate with extracts prepared from bacteria, fungi and nematodes. Furthermore, it seems likely that this substance is a normal constituent of sera from healthy fish. This could

therefore provide the fish with a potent defence against invading micro-organisms without there being a lag-phase for induction to take place.

An important problem in assessing the results obtained from experiments in fish physiology lies with the technique of husbandry, for the nature and the effect of suboptimal conditions are poorly understood; for instance little is known of the effect of population density, type of diet, handling techniques, nature of water flow and nature of the tank bottom, especially for burrowing species. Husbandry regimes may be stressful and cause a reduction in antibody production on challenge with an antigen, while at the same time increasing stress-induced non-specific substances like interferon and mammalian CRP. Certainly as techniques have improved for maintaining fish in laboratories, many more species have been shown to be immunologically reactive when they were suspected not to be so before, with the notable example of the hagfish, which was until very recently believed to be below the phylogenetic level of adaptive immune capacity.

The prospects for immunological studies in the lower vertebrates are exciting especially with the interest today in the evolutionary and phylogenetic aspects of immunology. Zoologists and medical immunologists are cooperating and the knowledge gained from the evolutionarily simpler systems may well have considerable influence on the understanding of the more complex mammalian counterpart.

A. E. E.

FOETAL ABNORMALITIES

Detection of Defects

from a Correspondent

SINCE many countries have relaxed their abortion laws there was a great deal of interest at the Fourth International Conference on Birth Defects (held under the auspices of the National Foundation March of Dimes in Vienna from September 2-9) in methods for the early prenatal detection of birth defects.

J. W. Littlefield (Massachusetts General Hospital, Boston) and C. Valenti (State University of New York, Brooklyn) emphasised the value of early amniocentesis in suitable pregnancies, especially for the detection of foetal chromosomal anomalies and to a much smaller extent biochemical errors. The apparent safety of early amniocentesis was shown by the results of a questionnaire survey carried out in the United

States; in more than 1,600 amniocenteses only ten errors of diagnosis were reported and the maternal and foetal morbidity attributed to this technique was almost nil. Spontaneous abortions following amniocentesis were less common than would be expected for this stage of pregnancy.

Some means for obtaining samples of foetal skin or blood are needed if many more biochemical errors are to be diagnosed *in utero*. This applies particularly to the common haemoglobinopathies. The use of the fibre optic foetoscope would be of great value in allowing samples to be obtained under direct vision, but J. B. Scrimgeour (University of Edinburgh) made it clear that the difficulties (and hazards) of foetoscopy hardly justify the routine use of this technique. By contrast S. Campbell (Queen Charlotte's Maternity Hospital, London) was very optimistic about the expanding use of ultrasound detection of foetal abnormalities (although the foetus is rarely still *in utero*), making it difficult to get a good picture), believing that it will be possible to diagnose some cases of spina bifida, congenital heart and renal disease as well as to measure foetal urinary output by successive measurements of bladder size. The value of serial measurements of biparietal diameter in detecting low growth potential as an indicator of, for example, chromosomal abnormalities was stressed. Ultrasound is non-invasive and has no known hazards at the intensity and pulse length used. D. J. H. Brock (Western General Hospital, Edinburgh) discussed his empirical but pragmatically crucial discovery that amniotic alpha foeto-protein levels are increased in conceptuses with neural tube defects.

Population screening and subsequent counselling aiming to prevent marriage between carriers of the same genetic disease may prove irresistible to public health authorities seeking a sequel to the successful control of epidemic infectious disease. But the experiences of G. Stamatoyannopoulos (University of Washington, Seattle) should prove a deterrent to excessive zeal, as even a well planned programme involving the sickle cell trait in Greece resulted in the stigmatisation of families following the collapse of marriage plans and the false belief among carriers that they were diseased and it achieved little or nothing in the way of actually reducing the number of heterozygotes marrying.

Mandatory screening, such as has apparently been imposed by some of the States in the United States on blacks, is to be condemned. By contrast M. M. Kaback (Harbor General Hospital, Torrance, California) described the successful screening programme for detecting carriers of Tay-Sachs disease among Askenazi Jews in the United States by

ASTRONOMY

Comet Kohoutek



THIS photograph of Comet Kohoutek was taken by the Royal Greenwich Observatory, Herstmonceux, at 05.00 h on Tuesday (November 6) using a 6-inch diameter lens at $f/4.5$, exposed for 8 minutes on a Kodak II a-O plate. The comet is clearly distinguished from the surrounding stars by its fuzzy appearance, and in particular by its tail, which extends away from the Sun. The scale of the photograph is indicated by the line which represents the radius of the full Moon. When the photograph was taken the comet was 182 million miles from the Earth.

The speed of the comet relative to the Sun was 22 miles per second, so that, during the 8 minutes' duration of the exposure, the comet moved more than 10,000 miles closer to Earth. Recent forecasts suggest that, at its brightest, the comet will be about as bright as the brightest star, Sirius. It may therefore not be bright enough to see in daylight, but should be easily visible in the twilight sky.

finding reduced levels of heat labile hexosaminidase A in serum and leukocytes. Where both husband and wife were deficient, antenatal diagnosis was carried out (in five pregnancies so far) and one foetus was found to have no heat labile hexosaminidase A activity, and was aborted. The success of the Tay-Sachs programme can be attributed to the education level of American Jews and to the availability of an effective means for antenatal diagnosis and induced abortion when necessary. The general value of genetic counselling was upheld by C. O. Carter (MRC Institute of Child Health, London) provided that it is non-directive and follows the initiative of patients rather than doctors.

J. Edwards (Birmingham Maternity Hospital) stressed the non-eugenic nature of genetic counselling conceding its value in assisting individual families.

Genetic engineering was discussed widely. P. A. Marks (Columbia University) described the use of avian myeloblastic virus reverse transcriptase in the production from natural mRNA of DNA coding for alpha and beta globin chains. The major portion of globin structural genes were synthesised and could in turn be used in heterologous cell free systems or intact oocytes to synthesise the appropriate globins. C. Merrill (National Institute of Mental Health, Bethesda) described possible means of introducing new genetic material into intact cells using the examples of prokaryotic genetic exchange. In a workshop on "Advances of Treatment of Hereditary Metabolic Disease" A. G. Bearn (New York Hospital, Cornell Medical Center) reviewed the use of organ transplantation, particularly the apparently successful use of renal allotransplantation in Fabry's disease, but was quite properly cautious about advocating liver transplants in Wilson's disease treatable by less heroic methods.

Of particular interest was the suggestion by P. E. Polani (Guy's Hospital, London) that mucopolysaccharidosis might be controlled by small skin grafts. H. W. Rudiger (University of Hamburg) described the 'Hunter corrective factor' and discussed how it might be produced from urine for therapeutic purposes. It is curious that earlier pyrogenic samples were most efficient in inducing mucopolysacchariduria.

R. G. Edwards (University of Cambridge) reviewed advances in reproductive biology and their implications and discussed the partial success achieved in the selection of X or Y bearing spermatozoa and *in vitro* fertilisation of human ova. There is no doubt that meiotic and early post-fertilisation studies would greatly assist in explaining non-disjunction and polyploidy. It is astonishing how much interference morulae and blastocysts will tolerate and still (in the mouse) develop into a normal foetus. It had proved possible to disaggregate and mix cell lines for study and to cut embryos in half using one half for study and the other half after implantation develops normally. The rarity with which malformed foetuses resulted from the implantation of animal ova fertilised *in vitro* will no doubt encourage those who would do the same in humans.

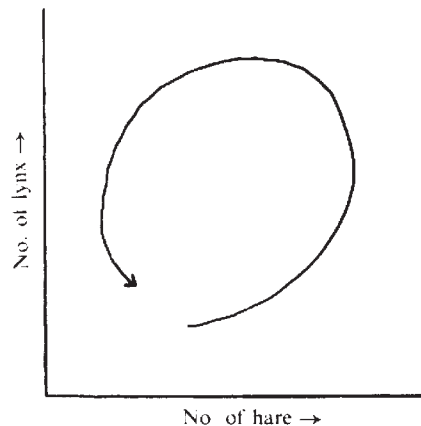
The moral and ethical issues in biomedical research were brilliantly reviewed by A. G. Mutoulsky (University of Washington) who avoided most of the clichés in considering the implications of the present rapid expansion of technology.

POPULATION ECOLOGY

Hare-Eats-Lynx Effect

from our Animal Ecology Correspondent

MOST biologists are familiar with the concept of population cycles and in particular with the well known and often quoted Canadian lynx-snowshoe hare interaction (MacLulich, *Univ. Toronto, Stud. Biol. Ser. No. 43; 1937, inter alia*). The provisions of the Lotka-Volterra equations, developed to describe the dynamics of predator-prey interactions, dictate that peaks of predator density must, temporarily, succeed peaks of prey density. When this dynamic arrangement is depicted according to Rosenzweig and MacArthur's graphical predation theory (*Amer. Nat.*, **97**, 209; 1963), with the numbers of predator on the ordinate and numbers of prey on the abscissa, the representation of one complete cycle of interaction is a quasi-circular figure progressing in a clockwise direction (see figure).



It comes as a considerable surprise to read the results of Gilpin's subjection of MacLulich's data to Rosenzweig and MacArthur's graphical analysis (*Amer. Nat.*, **107**, 727; 1973). He reveals that, for the 30 years 1875-1906 at least, the figure follows an anti-clockwise direction. This presents a severe interpretational problem because hares simply do not eat lynx. Other 10-year interactions, however, had the expected phase relationship. Gilpin and his colleagues (*Theoretical Population Biology*, in the press) have developed a predator-prey model in which growth rates of low density single species, interspecies coupling constants, intraspecific social interactions and intraspecific interference are considered. This model is much more flexible than the Lotka-Volterra one since it assumes that intraspecific factors are important. When the 30-year lynx-hare data in question were examined by this model the regression fit was poor. So poor that the signs of interspecies coupling constants were reversed. The model reinforced the findings of the graphical analysis that the hare was the predator. How, then,

can the anti-clockwise figure be explained?

Gilpin argues that hares could "kill" lynx if, for example, they carried a disease to them. He investigated the possibilities of this by writing an "epidemic" into his model which took effect only when a certain threshold density of hares had been reached. The "disease" was non-fatal to hares but took its toll of lynx. The results of the "epidemic" on the interaction when expressed graphically closely resembled the plot of the interaction during the 30 unexplained years. This explanation seems to be plausible but for the fact that no such disease has been found to occur in reality. Epidemic disease fatal to hares but not to lynx has, however, been identified (Chitty, *J. Anim. Ecol.*, **17**, 39; 1948).

The uniqueness of the series of data on the lynx-hare interaction lies in the number of years over which it was collected. Methodically logged by the Moravian Mission in Ungava since early in the last century, the figures for both species are the number of those trapped, skinned and put up for sale. Perhaps Gilpin has hit the nail on the head when he considers that fur trappers themselves are that elusive "disease". During lean years they turn their attention to chores other than trapping and head for the trap lines only when hares are again abundant. If, once at the lines, they turn a disproportionately large amount of their time to lynx trapping (which is infinitely more profitable and therefore reasonable to assume), they would return bags not inconsistent with the data that produce the curious hare-eats-lynx effect.

MacLulich's classic data may constitute the largest series of its kind on record but, as an indicative statement of actual population densities, their validity may be seen by this model to be in doubt. The model may yet be found to be of widespread ecological use, but the test of it will be as good as the test data.

AIR POLLUTION

Ozone and Wild Plants

from our Plant Ecology Correspondent

MOST of the concern surrounding air pollution in western Europe has centred on sulphur dioxide. In North America ozone has long been recognised as an important phytotoxic pollutant produced in the lower atmosphere by photochemical oxidation of certain products of combustion, especially nitrogen dioxide. The production of ozone is therefore particularly prevalent in urban environments where fossil fuels are combusted in large quantities, especially by automobile engines. Nitrogen dioxide formed in this process is split by ultra-

violet light to nitric oxide and atomic oxygen. The latter combines with molecular oxygen to form ozone. In the Los Angeles basin of Southern California daily peaks of ozone concentration of 15 to 38 p.p.h.m. (parts per hundred million) are frequent in summer and autumn. Even the remote areas of North America often have background concentrations of about 2 p.p.h.m.

Although it is known that ozone is phytotoxic, the threshold concentrations at which toxicity becomes apparent are known for relatively few species. Symptoms of external injury include chlorotic and necrotic lesions, but long before these appear a variety of metabolic processes, including photosynthesis, mitochondrial activity and amino acid synthesis, are affected, as well as membrane permeability. The critical exposures which cause such damage have been examined for some crop and tree species, but little is known of the response of natural plant communities.

Treshow and Stewart (*Biol. Conserv.*, **5**, 209; 1973) now have data on the threshold levels of ozone resulting in injury symptoms for a variety of wild plant species. The plants were enclosed in portable fumigation chambers in the field within grassland and woodland habitats in Utah and were exposed to ozone concentrations of 15, 25, 30 or 40 p.p.h.m. for 2 h; they were examined immediately after the experiment and again 2 days later. The experiments were thus concerned with short term, acute ozone pollution rather than long term chronic effects.

Of the seventy species examined, several, including *Bromus tectorum* and *Populus tremuloides*, both important or even dominant species in some types of grassland and woodland in the area, showed visible symptoms of injury after just 2 h exposure at 15 p.p.h.m. ozone. Only five species showed no signs of injury after 2 h exposure to 40 p.p.h.m. ozone. This range and order of sensitivity to ozone agrees well with data already published on crop plants.

Ambient ozone concentrations were generally lower than 15 p.p.h.m. In clearings a level of 9 p.p.h.m. was occasionally reached, but beneath the tree canopy in woodland, values were often less than 1 p.p.h.m. This does not necessarily mean that vegetation was unaffected, since damage at a physiological level normally precedes morphological damage and occurs at lower levels than chlorosis and necrosis. It is also possible that reproduction and germination might be impaired by chronic, low level pollution, which would result in long term changes in the composition of the vegetation.

Treshow and Stewart consider it possible that in time sensitive species may be eliminated, leaving unoccupied

niches and bare ground, which would result in a loss of stability in the vegetation. This is an unlikely event since there are several very resistant species in the communities, including some woody perennials (for example, *Acer grandidentatum*). Changes in vegetational composition could, however, have other repercussions such as the reduction of the productivity of an ecosystem.

BURROWING ANIMALS

Airing the Prairie Dog

from our Animal Behaviour Correspondent
PRAIRIE dogs (*Cynomys ludovicianus*) have a problem. These rodents live in long narrow burrows (about 12 cm wide and up to 30 m long) which must somehow be ventilated. Vogel, Ellington and Kilgore (*J. comp. Physiol.*, **85**, 1; 1973) have calculated that, at the very least, there must be complete exchange of the air in the burrow with the atmosphere every 10 h and probably more often. They found that neither diffusion of oxygen through the soil or at the burrow entrance nor the direct action of the wind adequately explains how gas is exchanged with the atmosphere to meet the respiratory needs of the animal. How then do the prairie dogs avoid suffocation?

Vogel *et al.* argue that two physical principles may underlie the ventilation. One is Bernoulli's principle in which

flow is related to pressure differences generated by variations in velocity along a streamline. The other is 'viscous sucking', the attraction of stagnant fluid to adjacent rapidly moving fluid. Either mechanism demands that the two entrances to a burrow be dissimilar. Now it has been known for some time that prairie dogs do indeed build two sorts of entrance to their burrows: 'dome craters' (wide and rounded) and 'rim craters' (narrow, steep-walled, with a rim). But the functional significance of these two types was unknown.

Investigations showed that the typical burrow in a prairie dog town has a rim crater at one end and a dome crater at the other. By dropping smoke candles down some burrows, it could also be shown that when even a slight breeze blew, air entered the burrow through the lower mound and left it through the higher one and that the air in the burrow was completely changed in less than 10 min. They then made scale models of prairie dog burrows in which the height and shape of the mounds at each end could be altered, and found that adequate ventilation would result if the mounds at each end of the tunnel were different either in shape or in height. Real mounds are different in both height and shape, which enhances their effectiveness. The authors point out that this ventilation is efficient almost regardless of which direction the wind is coming from and works even when there is very little wind at all.

Positive Control of Enzyme Synthesis

THE appearance of three articles on the control of repression of the tryptophan operon (*Nature new Biol.*, **245**, 131, 133 and 137; 1973) leaves no doubt that negative control of enzyme synthesis exists. More recently (see *Nature*, **245**, 407; 1973), it was suggested that positive control for biosynthetic schemes must also exist, and indeed that is precisely what Levinthal, Williams, Levinthal and Umbarger put forward in *Nature New Biology* next Wednesday (November 21).

Levinthal *et al.* have isolated a mutant in *Escherichia coli* K12 that renders the cells sensitive to leucine. The system being examined is the biosynthesis of isoleucine, valine and leucine, so it is no surprise that the enzyme threonine deaminase (specified by the *ilvA* gene) is involved (see *Nature new Biol.*, **244**, 34; 1973). Moreover, the *leu^s* mutation has been located within the *ilvA* gene, and it has been found to affect the regulatory properties of threonine deaminase with no concomitant alteration in its catalytic activity.

This unique class of *ilvA* mutant (note that *ilvA* encodes the enzyme threonine deaminase, the first enzyme in isoleucine biosynthesis, and is feedback-inhibited

by isoleucine) affords the putative regulatory role of threonine deaminase to be separated from its catalytic function in isoleucine and valine biosynthesis. The regulatory role not only involves the *ilvADE* operon and the genes *ilvB* and *ilvC* but also, predictably, is involved in the control of the amino acyl tRNA synthetases for isoleucine, valine and leucine.

Earlier Hatfield and Burns (*Proc. natn. Acad. Sci. U.S.A.*, **66**, 1027; 1970) had proffered a model to explain such regulatory properties inherent in threonine deaminase; they provided genetic and physiological evidence for the role of the enzyme in regulation of the pathway (at the level of synthesis). They suggested, however, that negative control may apply. Next Wednesday's paper by Levinthal *et al.* indeed confirms their idea that the *ilvA* gene product, strategically located, is a key regulatory target in the cell.

The *leu^s* mutant is found to be recessive to the wild type allele. This indicates the absence of function in the *ilvA* mutant and thus allows Levinthal *et al.* to aver that the wild type gene (*ilvA⁺*) produces a positive effector for the control of enzyme synthesis.

GRAVITATION

Radiation and Collapse

from a Correspondent

SYMPOSIUM 64 of the Extraordinary General Assembly of the International Astronomical Union, held in Warsaw on September 5–8 to commemorate the 400th anniversary of Nicholas Copernicus, reflected the growing interest in general relativity among astronomers and astrophysicists. Entitled "Gravitational Radiation and Gravitational Collapse" the programme ranged over most of the areas where it is believed that general relativistic effects are or soon will be susceptible of observation.

Pre-eminent among these areas is the question of the claims of J. Weber (University of Maryland) to have observed gravitational radiation. His colleague C. Misner reviewed the largely unsuccessful attempts of theoreticians to create models of the copious sources of gravitational waves required by Weber's claims. A short report by A. Poveda (University of Mexico) of a new (remarkably low) upper limit to the rate at which the Galaxy is losing mass of 1 solar mass per year, deduced from the orbits of globular clusters, will not make this task any easier. A. Tyson (Bell Laboratories) followed with an account of the current observational situation in which several groups have sought to repeat Weber's experiments with detectors which are claimed to be of equal or better sensitivity than Weber's 1969–70 antennae and have been unsuccessful.

An afternoon session was devoted to a discussion of these results between the parties involved. In particular R. Drever (University of Glasgow), P. Kafka (Max Planck Institute, Munich) and Tyson presented results which indicate that either Weber has not been seeing gravitational waves or that the source has weakened since 1970. On the other hand Weber made renewed claims concerning coincidences both between his own antennae and between his and those of D. Douglas (University of Rochester). The latter, which amount to some fifty-four zero delay coincidences above random in 7 days worth of data, represent an effect at the 2.7 standard deviation level. The fact that Douglas's instrument operates at 710 Hz would indicate that if this effect is real the signals really are broad band, as has long been suspected. It is hoped that the resolution of the puzzle will be brought about by further plans to exchange tapes and subject the raw data to a variety of signal processes, each favoured by one or other experimental groups. Of future plans perhaps the most striking was V. Braginsky's (State University of Moscow) idea of using a massive monocrystal instead of an aluminium bar with a resultant very

high quality factor to look for the sort of signals astronomers expect from nearby galaxies or gravitational bremsstrahlung produced by close encounters of stars—an idea emphasised by Ya. Zeldovich (Institute of Applied Mathematics, Moscow). Some encouraging progress was also reported on the supercooled detectors.

In the light of this it was amusing to hear J. Faulkner (Lick Observatory, California) suggesting that the effects of gravitational radiation might be found on our own doorstep, so to speak, in that mass transfer taking place in very short period binary star systems seems to be controlled in part by the radiation of angular momentum by gravitational waves.

Rather more positive were the sessions on collapse and black holes. R. Giacconi (American Science and Engineering) summarised the wealth of data from optical, radio and most importantly X-ray observation indicating that Cyg X-1 and SMC X-1 may possibly contain black holes. R. Kraft (Lick Observatory) was able considerably to strengthen the evidence by obtaining a lower bound to the distance to Cyg X-1 and hence a lower bound of $6 M_{\odot}$ to the mass of the

unseen secondary. There was, of course, much discussion of accretion models for such X-ray sources and this underlined the importance of numerical work by S. Teukolsky and W. Press (California Institute of Technology) on the stability of rotating black holes and although not everybody will agree with A. Starobinsky (Landau Institute, Moscow) that his ingenious analytical results on super-radiant scattering of waves off black holes, thereby increasing the energies of the waves at the expense of the black hole, guarantees that stability, they clearly represent important advances in black hole theory. The larger issue of "naked singularities" and whether a black hole can shed the respectable clothing of its event horizon to reveal the singularities inside in more extreme situations than those envisaged in the perturbation approach of Press, Teukolsky and Starobinsky were taken up by R. Penrose (University of Oxford) who provided a useful new definition of naked singularities and repeated previous warnings that the mathematical evidence against such objects is largely non-existent.

Among other topics discussed was the report by B. Carr (University of Cam-

Markers of CNS Malformation

SPINA bifida and anencephaly, two closely related malformations of the central nervous system (CNS), are comparatively common congenital disorders in the population of the United Kingdom, with a combined incidence of about 4 in every 1,000 births. Anencephalics die at birth, but after surgery, children with spina bifida live for a number of years with a high degree of physical and mental handicap. The policy of radical surgical intervention, which prolongs the lives of grossly incapacitated children and puts an enormous burden on their families, has recently been called in question.

A little over a year ago Brock and his colleagues (*Lancet*, ii, 197, 1252; 1972) reported that concentrations of α foetoprotein (AFP) were higher in the amniotic fluids of pregnancies leading to anencephaly and spina bifida. Their study was retrospective—that is, made on amniotic fluids obtained for other reasons and where the outcome of the pregnancy was already known—but it raised the hope of early diagnosis of these conditions followed by termination of the pregnancy. These hopes have now been realised in prospective studies (Lorber *et al.*, *Lancet*, i, 1187; 1973; Allan *et al.*, *Lancet*, ii, 522; 1973) and it is becoming an aspect of routine medical care to monitor pregnancies at risk for CNS malformations through AFP levels in the amniotic fluid. The report of Macri *et al.* in *Nature New*

Biology next Wednesday (November 21) adds a new dimension to these studies in that they have demonstrated a CSF-specific protein in the amniotic fluid of foetal Lewis rats with incomplete neural tube closures. Similar proteins are known in human CSF and, if they leak into the amniotic fluid, may provide another parameter to add to AFP in the early diagnosis of CNS malformations.

The real problem, however, in the antenatal diagnosis of spina bifida is identifying the at-risk pregnancy. Ascertainment through the previous birth of an affected child will allow detection of only a small number of cases, since more than 90% of such children will be born to mothers who have no relevant medical history to alert the obstetrician. Monitoring all pregnancies through early amniocentesis and protein analysis is clearly out of the question. A start to the problem has been made by Brock *et al.* (*Lancet*, ii, 923; 1973) who diagnosed an anencephalic pregnancy through AFP levels in the mother's blood. Presumably this foetal protein crossed the placental barrier from the amniotic fluid. The same is likely to occur with spina bifida, because amniotic fluid AFP is every bit as high. But before large-scale monitoring of maternal serum is contemplated it would be as well to have other markers of CNS malformation. The CSF-specific protein described by Macri *et al.* may be just such a protein.

bridge) on primaeval black holes formed very early on in the history of the Universe. It seems that these very small holes will not grow to gargantuan proportions (that is $10^{16} M_{\odot}$) as had been previously suspected by Zeldovich and Novikov. G. Gibbons (University of Cambridge) outlined some possible quantum mechanical effects associated with very small ($\ll 10^{-10}$ cm) holes, first proposed by S. W. Hawking (University of Cambridge) in collaboration with whom both the above pieces of work were carried out. Of special importance is the question of whether they can possess charge.

After this discussion of gravitational collapses it was peculiarly fitting that S. Chandrasekhar (University of Chicago), the man who in a sense started off the subject with the discovery of the upper limit to the mass of a cold degenerate newtonian configuration which bears his name, should have received the highest award of the Polish Academy of Sciences—the Smoluchowski medal—at this conference. It was also characteristic of his breadth of interests that it was for his work on Brownian motion that he received his award.

NUCLEAR PHYSICS

Continuum Shell Model

from our Nuclear Theory Correspondent

UNTIL fairly recently, nuclear structure and nuclear reaction studies developed almost independently, with occasional connections between the two. It was found most convenient to use a harmonic oscillator potential to generate the wave functions for shell model calculations because of its simple and useful analytical properties. These wave functions are indeed good approximations to the real ones, especially for light nuclei. But this potential arises to

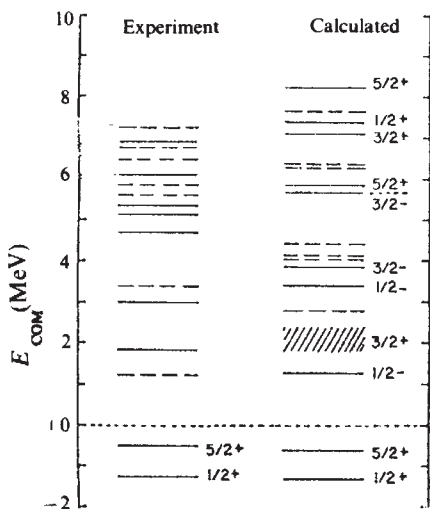


Fig. 1 Comparison between the calculated and experimental energy spectra of ^{15}C .

an infinite value outside the nucleus, which ensures that the nuclear wave functions soon fall to zero so that the nucleus is prevented from interacting with anything else.

Nuclear reaction studies, on the other hand, have most frequently used one-body potentials like the optical potential to represent the interaction between an incident particle and the nucleus. Such potentials are essentially energy averaged, so that the particular structural characteristics of each nucleus are largely washed out and the potential is almost the same for all nuclei. Thus, paradoxically, nuclear structure theory allowed no reactions, and nuclear reaction theory was almost independent of nuclear structure.

In particular, the shell model calculations, although very sophisticated, could only give the properties of bound states and states that are in reality unstable to particle emission had to be treated as if they did not decay. Reaction calculations, on the other hand, did not include important features of the structure of the interacting nucleus and were then unable to give an adequate account of the increasingly detailed experimental data. This barrier is now being broken down by nuclear structure calculations that include unbound or scattering particles and by nuclear reaction theories that take detailed account of the structure of the interacting nuclei.

A recent example of the former is the continuum shell model calculations of the structure of ^{15}C carried out by Philpott (*Nucl. Phys.*, **A208**, 236; 1973). This is a fully microscopic shell model treatment of the structure of ^{15}C taking account of continuum states as well as bound states and including configuration mixing, finite range and spin-dependent forces and using fully antisymmetrised wave functions. This gives not only the usual shell model states but also a wide range of resonance phenomena, including broad single-particle and narrow many-body resonance in both elastic and inelastic scattering.

To do this the total wave function is expanded as a sum of states, some localised and some describing the separation of a nucleon from the nucleus. If this is inserted into the Schrodinger equation the usual separation procedure gives a set of coupled differential equations for the bound and unbound wave functions, and these can be solved subject to the appropriate boundary conditions.

These equations include matrix elements depending on potentials that have to be specified explicitly before they can be evaluated. These potentials have one-body and two-body components. The one-body component represents the interaction between each active nucleon and the core, and is

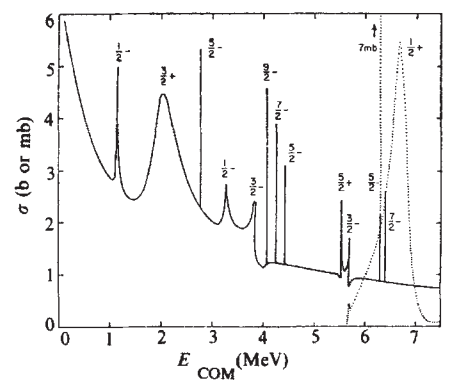


Fig. 2 Calculated total elastic and reaction cross sections for the interaction of neutrons with ^{14}C . —, σ_E (b); ---, σ_R (mb).

conveniently provided by a Saxon-Woods potential whose parameters are adjusted to fit the observed single-particle energies in ^{13}C . The two-body component represents interactions among the three active nucleons outside the ^{12}C core, and this was taken from a previous shell model calculation in which a standard central Gaussian two-body force was used to fit energy levels in ^{14}N and ^{14}C .

The resulting energy level spectrum (shown in Fig. 1) is naturally very similar to the conventional shell model for the bound states, but the unbound states now have widths corresponding to their decay probabilities. It is also possible to calculate the total elastic and reaction cross sections for the interaction of neutrons with ^{14}C ; these are shown in Fig. 2. There is a large variation in the widths of the states, depending on their individual structure. In addition, the form factors for single nucleon transfer reactions can be calculated within the shell model framework, since the wave functions behave correctly beyond the nucleus.

There is not yet sufficient experimental data on any one nucleus to make a detailed test of this theory, and indeed in its present form it is premature to expect more than qualitative agreement. Its importance is that it provides a way of unifying structure and reaction data, and further theoretical development, together with the use of more realistic forces, will give more reliable predictions that can be compared with new experimental data.

PALAEOMAGNETISM

Réunion Event Defined

from our Geomagnetism Correspondent

ALTHOUGH the boundaries of the major intervals (epochs) of the geomagnetic polarity-time scale for the past four million years or so are now fairly well defined, the number and precise ages of the much shorter polarity reversal events are still the subject of investiga-

tion and discussion. Part of the problem is that the shorter the event the smaller is the chance of detecting it, especially in continental igneous rocks whose sequences are not continuous in time; and even if an event is found to be recorded in more than one lava flow the relevant outcrops are likely to be stratigraphically unrelated. When to these difficulties is added the fact that the errors on potassium-argon ages are usually comparable to the duration of the events being measured, it is easy to see that events will be difficult to resolve. Nor are deep sea sediments likely to provide the final solution, because, as Watkins (*Bull. Geol. Soc. Am.*, **83**, 551; 1972) has pointed out, although sediments frequently form continuous time sequences, the magnetic data from them are not always too consistent and accurate dating methods are even less applicable than to igneous rocks.

The confusion likely to result is well illustrated by the polarity events within the Matuyama reversed epoch from 2.41 to 0.69 million years ago. One of the major normal events is undoubtedly the Olduvai event which was discovered in rocks from the Olduvai Gorge, Tanzania, and later also recognised in lavas from Alaska and the Cocos Islands as well as in deep sea sedimentary cores. The age assigned to the Olduvai normal basalts by Grommé and Hay (*Nature*,

200, 560; 1963) was 1.92 m.y., and so when Chamalaun and McDougall (*Nature*, **210**, 1212; 1966) found normal polarity lavas of ages 2.03 ± 0.05 m.y. (at the normal to reversed boundary) on Réunion island they tentatively correlated them with the Olduvai event while recognising that there was a significant age difference. Later, however, McDougall and Wensink (*Earth planet. Sci. Lett.*, **1**, 232; 1966) discovered another major normal polarity event (the so-called Gilsa event) at 1.6 m.y. in Icelandic lavas; and this was subsequently also recognised in Tenerife, Alaska and Madeira. Moreover, even more recently, Grommé and Hay (*Earth planet. Sci. Lett.*, **10**, 179; 1971) have revised the age of the Olduvai event to 1.72 m.y.

It now seems probable that the Olduvai and Gilsa events are identical, although the identity cannot yet be considered proved. But what is more certain is that the now-named Réunion event first recognised by Chamalaun and McDougall is quite distinct from both the Olduvai and Gilsa events. At the same time, however, the limits of the Réunion event have never been precisely defined; and so to remedy this deficiency, McDougall and Watkins (*Earth planet. Sci. Lett.*, **19**, 443; 1973) have returned to Réunion to study two sequences of olivine basalt lava flows which between them cover the full reversed-normal-

reversed polarity sequence defining the event.

The stratigraphically higher of the two lava sequences was sampled by the two lava sequences was sampled by four reversed flows overlying two normal flows and separated by a flow whose four specimens gave scattered directions of magnetisation. Since this stratigraphically intermediate flow produced fresh specimens petrographically similar to those from the flows immediately above and below, it seems likely that it was extruded during the normal to reverse polarity transition. The stratigraphically lower of the two lava sequences comprised sixteen reversed flows overlain by eleven normal flows, the whole being overlain by a flow of transitional polarity. The two lava sequences may thus even overlap slightly; but, be that as it may, they certainly record the full Réunion event.

From three potassium-argon dated flows in the upper sequence, McDougall and Watkins conclude that the normal to reverse transition defining the end of the Réunion event took less than 30,000 yr and that the absolute age of the transition is 2.02 ± 0.2 m.y. Similarly, from eight dated flows in the lower lava sequence, they estimate the reverse to normal polarity change at the start of the event to have occurred 2.01 ± 0.01 m.y. ago. Clearly, the lack of a significant age difference between the two transitions means that the Réunion event was very short, probably between 10,000 and 50,000 yr long. This shortness probably explains why the event has been recorded so rarely in deep sea cores, and in this, as well as in absolute age, it contrasts strongly with the much longer Olduvai event.

Finally, there has been some suggestion in the past that there may, in fact, be two distinct normal events around 2.0 m.y. ago. Normal polarity lavas in Alaska, for example, have been dated at 1.95 ± 0.10 and 1.96 ± 0.04 m.y., in Argentina at 2.05 ± 0.02 and 2.06 ± 0.03 , and on Cocos Island at 2.09 ± 0.04 . Insofar as all the errors involved here give ranges overlapping, or almost overlapping, the age range of the Réunion event, the corresponding rocks could represent the same event. Similarly the age of the Réunion event as determined by McDougall and Watkins corresponds closely to the age of 1.99 ± 0.06 or 1.97 m.y. for the W anomaly recognised in oceanic ridge magnetic profiles by Emilia and Heinrichs (*Mar. geophys. Res.*, **1**, 436; 1972). On the other hand, anomaly X, identified by Heirtzler *et al.* (*J. geophys. Res.*, **72**, 2603; 1957), has been dated at 2.3 ± 0.1 m.y. As McDougall and Watkins note, their results neither confirm nor refute the idea of a normal event pair; but data already in existence suggest that the polarity-time scale story is still far from complete.

CHEMISTRY

Dimeric Oxygen

from our Chemical Physics Correspondent
SINCE the ground state of an oxygen molecule contains two unpaired electrons, there has for many years been speculation as to whether the dimer, $(O_2)_2$, could form. In 1924 G. N. Lewis suggested, on the basis of measurements on the magnetic susceptibility of the liquid, that some dimerisation does occur, but most of the recent evidence does not support the presence of abnormal forces between two oxygen molecules. Now, from their spectroscopic studies on the cold gas, Long and Ewing (*J. chem. Phys.*, **58**, 4824; 1973) find clear evidence of dimer formation. Nevertheless they note that such dimerisation is of the type to be expected from a typical van der Waals attraction and does not seem to have any exceptional features associated with the unpaired electrons.

Because of its symmetry an isolated oxygen molecule shows no infrared absorption, although at high pressures some very broad features are observed and interpreted in terms of a loss of symmetry during a collision. The collisions are of short duration, so that the uncertainty principle predicts the ob-

served broadness. The new observation of weak, but much narrower, features on top of the band must indicate a longer lived complex. The most prominent features are at 1,553.3 and 1,557.9 cm^{-1} which is the correct position for the excitation of an O-O stretching vibration. The intensity is proportional to the square of the pressure, which indicates a dimer, and although the features are plain at 87 K they disappear above 100 K. Accurate temperature and intensity measurements indicate a molar energy of formation of -2.3 kJ mol^{-1} . The fine structure of eight lines in all has been qualitatively assigned to changes in the relative rotational motions of the molecules with a low hindering potential.

The electronic absorption centred at 17,282 cm^{-1} also has sharp features superimposed on its collision-induced broad line. These confirm the general picture and a fine structure analysis suggests a vibrational interval of 24 cm^{-1} in the ground state for the mode associated with the intermolecular distance coordinate. The value falls to 18 cm^{-1} in the electronically excited level and both are appropriate values for the weak minimum associated with ordinary van der Waals type intermolecular potentials.

Evolution of Insect Wings and Flight

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Disputes during the nineteenth century as to whether insect wings originated from gills or from paranotal lobes ended with almost universal acceptance of the paranotal theory. Sir Vincent Wigglesworth, however, here gives evidence that the thoracic styli were perhaps the precursors of insect wings, and describes a theory of the origin of insect flight.

THE earliest suggestion for the origin of wings in insects was that of Oken¹ who asserted that in the air-breathing Ancylozoa the external branchial lamellae to which the tracheae ran, hardened and were converted into wings. This view was adopted by Gegenbaur²; but a few years later Müller³ noted that the first instar larva of *Kaloterpes* had prominent expansions from the terga of the pro- and mesothorax, and in later instars from the metathorax. He assumed these to be the rudiments of wings; and since, when they first appeared they were devoid of tracheae, he argued from the principle of recapitulation that they could not have been derived from gills and therefore Gegenbaur must be wrong. Many other ideas are commonly attributed to Müller, but that was all he said.

Disputes over these two theories continued throughout the nineteenth century. The decisive paper seems to have been that of Crampton⁴; a highly judicial work in which twenty-one items of evidence are set out in support of the gill theory and twenty-one items of evidence in support of what Crampton named the paranotal theory. He pronounced judgment unequivocally in favour of the paranotal theory. Snodgrass⁵⁻⁷ in all his writings from 1927-1963 took the same view. At present there is almost universal support for this theory.

Packard⁸ had early suggested that the tergal expansions were first used in gliding, perhaps in a leaping insect. Hinton⁹ started further back and stressed the importance of attitude control of legs and body to ensure that a falling insect landed on its feet and could make a quick getaway from predators, notably spiders. Flower¹⁰, assuming that gliding began in a small insect about 1 cm in length, pointed out that even diminutive paranotal lobes would have been important in attitude control. Then hinges to the planes, and muscular control of their inclination, would lead first to control of the direction of gliding and finally to slow flapping flight¹¹⁻¹⁴.

Gill Theory

In working through the extensive literature I came to feel that the case for the gill theory had been too summarily dismissed. I was impressed by Woodworth's thoughtful paper, which took twenty years in the writing. Woodworth points out that gills are soft, permeable, floppy structures, singularly unsuited for conversion into wings operating in dry air. He argues that it is more likely that the wings should have been formed from some structure accessory to the gills and he

develops a case for the mobile gill plates on the abdomen of mayfly larvae being homologous with wings.

As Heymons¹⁵, Börner¹⁷ and others have shown, the gills of *Ephemera* and *Chloëon* are clearly derived from the segmental appendages of the abdomen. The abdominal appendages of Apterygota are represented by the styli. These are not in fact vestigial legs but the exopodites arising from rounded limb bases or coxites, the main appendages or telopodites of which have been lost. In *Ephemera* the appendages are feathered structures; the intrinsic muscles attached to the base of the styli of Apterygota become the muscles which vibrate the gill lamellae. It is in the free-swimming forms such as *Chloëon*, *Baëtis*, and *Ecdyonurus* that they become most wing-like. They migrate laterally and may even become articulated to the dorsum of the abdomen¹⁸.

The vibrating lamellae are not as a rule themselves the gills: they may be only weakly tracheated; they are often stiffened by veins; the leading margin is often rigid and furnished with hairs and spicules; the posterior margin more flexible. The respiratory component takes the form of tracheated filaments, or simply tracheated areas of the abdominal surface. The gill plates serve to drive currents of water over the respiratory surfaces, and may occasionally be used in locomotion.

In the bottom-living larva of *Caenis* the functional gills are limited to the dorsum of segments three to six of the abdomen. As Eastham¹⁹ showed they set up transverse currents across the abdomen. On the second segment gills are wanting, but there is a pair of remarkably wing-like articulated gill-plates (called 'pseudoelytra' by Eastham) which extend backwards to form a gill chamber for the functional gills. In other mayfly larvae similar gill chambers are formed by the backward extension of the wing lobes. Some mayfly larvae have gill filaments arising below the head and thorax²⁰, but wing-like gill-plates are confined to the abdomen. The *Caenis* larva has a little tapering structure, like an unchanged stylus, projecting laterally below the wing lobe; but this is in fact the vestige of the gill belonging to the first abdominal segment and it is articulated to the postero-lateral angle of that segment.

It is surprising that vibratile gill-plates in mayfly larvae should be confined to the abdomen. One would have expected them to be well developed on the thorax, which is the chief locomotor centre with major ganglia and extensive muscles. That is where the gills are always best developed in Crustacea, as in Euphausiidae. The explanation, of course, is that the thorax is already committed to the development of wings. Börner¹⁷ showed that the gill-plates of *Chloëon* arise from the modified limb-bases of the abdomen. In the thorax the limb-base has provided the pleural stiffening of the thoracic box which extends up to the attachment of the wing lobe. There are no gill-plates to be seen.

If there is any real relation between the wing-like gill-plates of the abdomen and the wings of the thorax it must be sought in the non-existent ancestors of the pterygote insects. Fossils of these have not yet been found. The best we can do is to look at the Thysanura, which are the nearest relatives we have of the early pterygotes. *Petrobius maritimus*, for example, is something like a living fossil; it clearly missed the boat, and has been sitting around on the rocks by the seashore since Devonian times. In *Petrobius* and other

machilids, most of the abdominal segments have styli; and they have serially homologous styli of the same character on the meso- and metathorax, the segments on which the wings of Pterygota develop. I suggest that the thoracic styli of machilids were the precursors of insect wings.

Thoracic Styli

The styli of the thorax in *Machilis* arise from the base of the coxa. They have played an important part in the arguments of morphologists. It is because of their survival in *Machilis* that the abdominal styli have been identified as the exopodites of serial appendages of which the limb-base remains but the main limb or telopodite has been lost^{5,21}. Exités and endites of the proximal limb segment of arthropods, from trilobites to insects, have an extraordinary history. They have furnished most of the remarkable tools of the phylum: mandibles and other mouth parts; gills of trilobites and Crustacea; swimming and grasping appendages; gill-plates of Ephemeroptera; styliform gills of Neuroptera, Megaloptera and Coleoptera; specialised parts of the external genitalia. Their evolutionary potential is comparable with that of the vertebrate limb.

There seems no reason why a primary apterygote, adapting to aquatic life, should not have developed gills as in the abdomen of Ephemeroptera. Such an insect would have suffered no phylogenetic inhibitions about developing gill-plates from the styli of the meso- and metathorax. In the course of this process, if the changes in the abdomen of mayflies are any guide, the gill-plates would have migrated towards the dorsum and the articulated bases would have become approximated to the terga. It is of interest that certain of the symphylids carry styli on the base of the second and third pairs of legs, and these styli can become incorporated into the cuticular wall of the segment²².

Although the pleuron in insects is believed to be derived from the limb-base, there is no sign of the recapitulation of this process during embryonic development. It is therefore unlikely that we should get evidence in this way of the coxal origin of wings. The most we can hope for is to see the point of origin of the wing rudiment. Tower²³ looked carefully into this question. He showed that in *Periplaneta* the epidermal thickenings, which form the wing lobes, arise on the sides of the thorax at exactly the same sites as in Coleoptera and Lepidoptera; "but (he writes) the wings soon migrate dorsally and posteriorly, and when they become external appear as direct backward projections of the tergum". Tower could thus find no support for the paranotal origin of wings.

The rather weak muscles which actuate the abdominal gill-plates of mayfly larvae, which are derived from the muscles of the styli, retain their attachment to the coxal or subcoxal limb-base. Corresponding muscles would doubtless have been retained by thoracic gill-plates (although the thoracic styli of existing Thysanura, like the corresponding styli in Symphyla, do not in fact retain their muscles). Such a muscular supply would explain why the two most important direct flight muscles in primitive insects (such as grasshoppers) have their origin in the coxa and run all the way through the thorax to be inserted into the basal sclerites of the wing.

Börner¹⁷ established the homology between the gill bases and the thoracic subcoxae, and showed that the tracheae supplying the gills correspond with the tracheae of the thoracic legs. It is interesting that his figure shows the single trachea, which supplies the wing lobe of *Chloëon*, arising from the leg trachea as it runs towards the coxa.

It is likely that thoracic gill-plates would have been supplied by more powerful muscles, and would be larger structures, than the abdominal gills of mayflies. Lubbock²⁴, who accepted the branchial origin of wings, did not consider that the branchiae of *Chloëon* are used in swimming; but he

claimed that in earlier forms, thoracic branchiae might well have been used for locomotion under water. As Lubbock pointed out, the chalcid wasp *Polynema natans*, as he himself discovered, rows its way under water by means of its wings. Although he did not for a moment suggest that this had any direct connection with the origin of flight, it did serve to show that the same wings could be used for locomotion in the air and under water.

It surprises me that the hypothesis here advanced, that the thoracic styli of Apterygota were the precursors of insect wings, which seems a rather obvious suggestion, seems never to have been put forward before.

Origin of Flight

Ten years ago I put forward a 'dispersal theory' of the origin of flight²⁵, without considering the morphological origin of wings. I now attempt to re-state that theory with special reference to the hypothesis that winged insects arose from a secondarily aquatic ancestor.

The existence of a vast aerial plankton of insects and other arthropods extending up into the sky for at least 14,000 foot is well established. Most of these are winged forms; but wingless Thysanura, Collembola, young stages of Hemiptera and Orthoptera, larvae of Coleoptera, Lepidoptera and Diptera and vast numbers of wingless ants have been captured at heights up to 5,000 foot²⁶.

In our postulated aquatic insect, stranded by the drying out of its breeding places, the vibratile gills, even if used for locomotion under water, could not be used directly as propellers in the air; but they would be pre-adapted for development by the insect of the necessary skills, to increase its buoyancy and keep afloat. I picture a small aquatic insect, in the range of 0.5–1.5 cm, as in existing Thysanura, with mobile thoracic gill-plates, which would thus learn to fly while passively air-borne; not, of course, by studying aerodynamics, but by trial and error.

Such an insect would have had no difficulty in adapting to the dry atmosphere. Judging by existing aquatic insects, the integument could well have retained its waterproof properties²⁷. Existing mayfly larvae use their spiracles solely for moulting²⁸; they are not functional in respiration on exposure to the air. In Odonata larvae, however, some of the spiracles do remain functional in aerial respiration. That could well have been so in our aquatic prepterygote. The long slender cerci of Thysanura, as Leston²⁹ has pointed out, could have been an asset for flight stability, as indeed, in conjunction with the long forelegs, they are in existing Ephemeroptera.

The dispersal theory fits in well with our knowledge of the ecology of flying insects. The central idea which I put forward in 1963 was stimulated in part by the work of Kennedy³⁰ on locusts, of Johnson³¹ on aphids and on Rainey's description of dispersal in the desert locust, which is carried by converging winds and lands where the rain falls³². More recently, both Leston²⁹ and Rainey³³ have favoured the idea that insect flight originated in an arid climate. The climate during Devonian times would have provided the conditions needed for this evolution. The combined Lower, Middle and Upper Devonian occupied some 65 m.y. We read about evidence of torrential rains, of extensive swamps, of arid conditions and the formation of aeolian sandstones and wind-driven layers of sand between sedimentary deposits.

Mamajev³⁴ considers that the Devonian insects were associated with the vegetation in the swamp forest along the banks of lakes and that they were probably not true terrestrial forms but followed a semi-aquatic or amphibious mode of life. It would be indeed surprising if, in the course of 50 m.y., the early hexapods had failed to colonise the inland waters. These aquatic animals would presumably feed like mayfly larvae today: by scraping up the algae and diatoms which must have abounded on submerged stones and vegetation. It is likely that they built up gigantic populations, like

mayflies, so that enormous mortality could be tolerated by the stranded migrants. As Rainey³³ has emphasised, arid and semi-arid areas are characterised by vigorous thermal up-currents developing regularly by day over heated ground and rising at speeds of several m s^{-1} . Insects would be caught up also in whirlwinds and dust-devils and carried up into rain clouds.

It is well to recall the parallel theory of Romer³⁵ which describes the evolution of terrestrial locomotion in crossopterygian fishes in their efforts to crawl out of one drying pool during the seasonal droughts of the late Devonian, and hobble over land to another pool with water in it. Walking limbs arose, not in an effort to colonise the land, but in order to survive in water. Likewise in the aquatic ancestors of winged insects: it is suggested that wings arose to make possible a return to water.

I claim, therefore, that the dispersal theory of the origin of insect flight, most of the elements of which have been put forward at one time or another during the past hundred years, offers the least strain to the imagination, because, as Southwood³⁶, Rainey³², Johnson³¹ and others have pointed out, most of the processes involved have been seen in operation around us to the present day.

This article is a summary of a contribution to the Symposium on Insect Flight organised by the Royal Entomological Society of London, September 20–21, 1973:

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Nitrogen Dioxide Concentrations in the Atmosphere

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Nitrogen dioxide and its related compounds react strongly with ozone, usually resulting in the destruction of the ozone, and life seems to be delicately adjusted to the radiation shield which the stratospheric ozone provides. We have found that atmospheric NO₂ occurs in two layers, described here, which have very different properties.

NITROGEN dioxide strongly absorbs visible and near ultra-violet light and for wavelengths between 0.43 μm and 0.45 μm its absorption changes rapidly with wavelength. This permits the measurement of atmospheric NO₂ by measuring its absorption of sunlight, using methods similar to those which have been used for ozone for more than 50 yr (ref. 1). We

have adapted a photon counting spectrophotometer² which was originally designed for ozone measurements for this purpose.

The spectrophotometer measures the intensities at wavelengths $\lambda_1 = 0.4377 \mu\text{m}$, $\lambda_2 = 0.4448 \mu\text{m}$, $\lambda_3 = 0.4500 \mu\text{m}$ with slit widths equivalent to about 0.3 nm. At these wavelengths, and for our slit widths, the solar spectrum is at stationary points and strong differential absorption by NO₂ is achieved. If I_1 is the measured intensity at λ_1 and so on, then after the three intensities have been measured, the function, $F = \log_{10} [I_1/I_2] - 1.46 \log_{10} [I_2/I_3]$, is calculated [$1.46 = (\lambda_2 - \lambda_1)/(\lambda_3 - \lambda_2)$]. This function automatically eliminates all effects for which the absorption at the three wavelengths is linear with wavelength. Ozone absorption is reduced to an extent where it cannot cause interference. Rayleigh scattering, though perhaps not totally removed, is reduced to an unobservable effect. On the other hand, a layer of NO₂ (at STP) 10 μm thick will increase this function by 0.007; which we can measure quite reliably, except when intensities are extremely low. We do not know of anything else with sufficient, non-linear absorp-

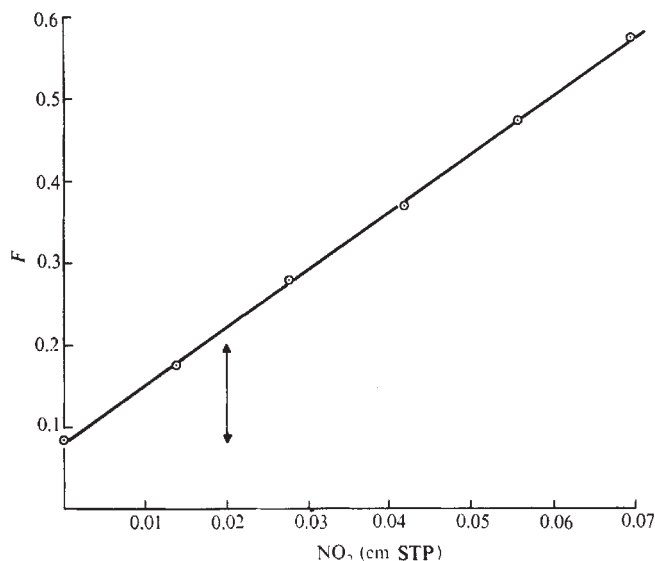


Fig. 1 The growth of absorption by NO_2 , as measured by our spectrograph. The light source was a quartz halogen lamp. To reduce association into N_2O_4 the NO_2 was dispersed in air at room temperature and pressure in a column 30 cm long. F is defined in the text. The vertical line gives the range of optical absorption observed. The slope of the line is 7 cm^{-1} .

tion to interfere. If we displace all our wavelengths by a small amount we can reverse the effect of NO_2 on our function F , though the measurements become less sensitive. The effects we observe are then quantitatively reproduced but with reversed sign as would be expected.

With amounts of NO_2 comparable with those observed in the optical paths attained in the atmosphere we have confirmed the exponential growth of absorption, which has been reported by others³, and also we have measured the value for the effective absorption coefficient, which is consistent with published values (Fig. 1).

Following the analogy with ozone measurements, we have made measurements of the three intensities in direct sunlight and also in the light scattered from the clear zenith blue sky. When these are made for changing solar zenith angles and especially during the periods near sunrise or sunset, when the Sun is low or below the horizon, useful information can usually be obtained about the amount and vertical distribution.

Observations from the ground in Southern Ontario near Picton at 44°N , 77°W , in conditions of clear air with a visibility greater than 30 km and with a cloudless sky, give measurements of which Fig. 2a and b are typical. The differences between sunrise and sunset are persistent and during the first half of 1973 the sky curves have not changed to a detectable extent.

Low Level NO_2

At large solar zenith angles the direct Sun curve is separated from the zenith sky curve because of the presence of NO_2 near the ground, which, when the Sun is low, usually has a considerably higher concentration than exists in the upper air. This may be seen as follows: the light from the direct Sun passes obliquely through the lower layers and their optical effect is enhanced in accordance with the secant of the zenith angle. On the other hand the light from the clear zenith sky is scattered downward by air at rather high levels and it passes through the low level NO_2 vertically, the absorption of which does not become increased by obliquity. We usually assume this low level NO_2 is below 2 km. Deep convection may carry it upward into higher layers but on the clear days when we take our measurements this will not usually be the case. So long as it is below 10 km it will not alter the conclusions we reach in this paper regarding stratospheric NO_2 .

We can also measure the low level NO_2 by climbing through

it and observing the direct Sun while we do so. We have only two measurements of this kind. One made at noon with a solar zenith angle of about 27° (Fig. 7) when we observed very little NO_2 in the lowest two or three km, less than we could measure with certainty, and much less than was measured at sunrise and sunset on the same day. The second measurement was made in the late afternoon with a solar zenith angle of 75° . The quantities found were less than we typically find at sunrise or sunset but were measurable in amount. The actual concentrations found, assumed, in the case of the observations made from the ground, to be distributed through the lowest 2 km, are shown against the appropriate layer in Fig. 4.

The amount is quite variable but it is subject to a strong diurnal variation. There is very little when the Sun is high. There is on the average about 50% more at sunrise than at sunset. We have made observations on the direct Moon; they have low accuracy but suggest that night time amounts of NO_2 are similar to those observed at sunrise.

NO_2 in the Upper Atmosphere

The chief interest centres on the NO_2 in the upper atmosphere and in the stratosphere. We had hoped to obtain this information from the shape of the 'zenith sky' curves, particularly from that part obtained during twilight. This technique is in routine use for deducing ozone vertical distributions and the methods used for inverting the curves have been widely discussed⁴.

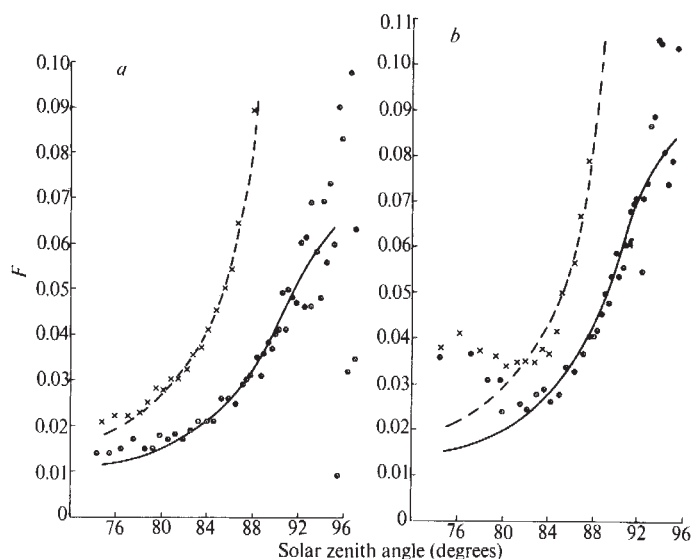


Fig. 2 a, Clear morning observations, July 23, 1973, Picton, Ontario. $\times$, Observed direct Sun; $\circ$, observed zenith sky. ---, Calculated direct Sun; —, calculated zenith sky; both for the sunrise distribution shown in Fig. 4 and using the F_0 as derived in Fig. 6. b, Clear evening observations, July 23, 1973, Picton, Ontario. $\times$, Observed direct Sun; $\circ$, observed zenith sky. ---, Calculated direct Sun; —, calculated zenith sky, both for the sunset distribution shown in Fig. 4 using the F_0 as derived in Fig. 6.

Unfortunately, there is ambiguity about the vertical distributions of the upper atmosphere NO_2 which would produce the observed zenith sky curves. They would be produced either by NO_2 which was almost wholly above 30 km with very little below, or by a suitable NO_2 density at all levels above a few km which changed only slowly with altitude.

The way the ambiguity arises is as follows: it is well known that in the ozone case, the zenith sky curves show an 'Umkehr' and if a well defined layer of NO_2 existed our zenith sky curves would be expected to turn down as the mean scattering level

enters the NO_2 layer and then turn up again when the Sun is below the horizon and the effect of the sunlight passing through the upper part of the layer becomes important. This latter effect which would not arise on a flat Earth is the so called 'second Umkehr'. We observe no vestige of an Umkehr of any kind. This may be because (a) the mean scattering level never enters the NO_2 layer, which, calculation shows, requires that all the NO_2 be above 30 km or (b) there is no layer; that is to say the NO_2 is approximately uniformly distributed through the atmosphere. We do not know of any other way in which the Umkehr can be avoided.

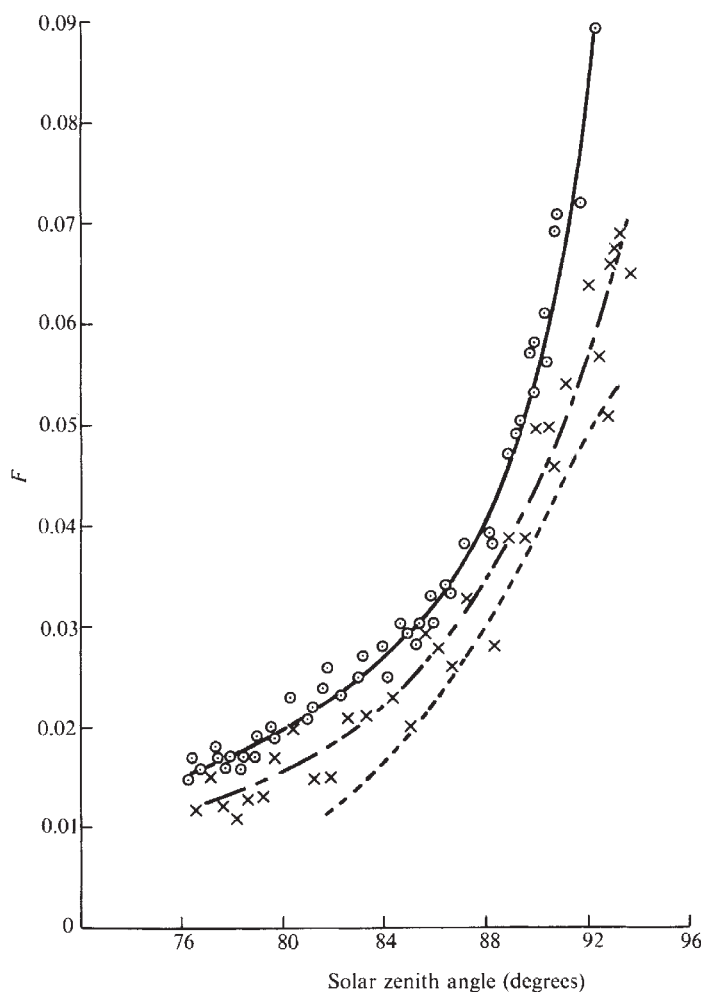


Fig. 3 Observations from an aircraft (12 km), June 13, 1973, Val d'Or, Quebec. $\circ$, Observed direct Sun sunset; $\times$, observed zenith sky sunset; —, mean direct Sun sunset; - - -, mean zenith sky sunset; - - -, mean direct Sun sunrise.

Sunrise and Sunset Observations

To resolve this, measurements similar to those described above were made at a height of 12 km at 48°N , 78°W . Because of the height of the observation, the direct Sun could be observed to zenith angles greater than 90° ; this gives the benefit of greatly amplifying the optical path where it is tangential to the Earth, and enables good local concentration measurements to be made at the flight level and below it. The quality and nature of the observations made from the aircraft may be seen in Fig. 3 which shows the observations made at sunset, together with smooth curves drawn through the points. For the morning measurements only direct Sun measurements are available. They are shown in Fig. 3 with data points omitted for clarity.

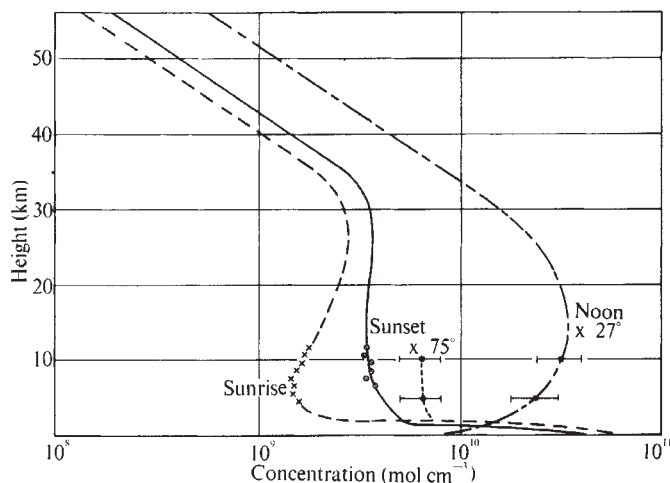


Fig. 4 Vertical distribution of NO_2 in the atmosphere at different times of the day, as measured over Southern Ontario in first half of 1973.

The direct Sun measurements give absolute concentrations between 6 km and 12 km valid for the instant when the Sun is at a zenith angle of 90° . The accuracy and dependability of these values seem high. They are shown as points on Fig. 4. These observations exclude the possibility of all the upper atmosphere NO_2 being above 30 km with very little below.

The deduced upper atmosphere distributions at sunrise and sunset are then as shown in Fig. 4. These distributions are consistent with all the dusk and dawn observations whether from the air or from the ground. The upper air distributions are necessarily strongly smoothed and are very uncertain above 45 km. In Fig. 5 we show the same data plotted as mixing ratio rather than as concentrations. Each layer contributes most to the absorption at the period when the Sun rays are tangential to the Earth; therefore the curves effectively relate to the instant of sunrise or sunset.

The noontime NO_2 is difficult to measure. The presence of a diurnal variation excludes the use of the normal method. This would be to plot the value of F against the secant of the solar zenith angle ($\sec \chi$). The points should lie on a straight line, the slope of which would be the change in F for vertical transmission and would give the total NO_2 overhead. For the whole day the relation between F and $\sec \chi$ is not linear as is seen in Fig. 6. But near sunset (or sunrise) $\sec \chi$ changes

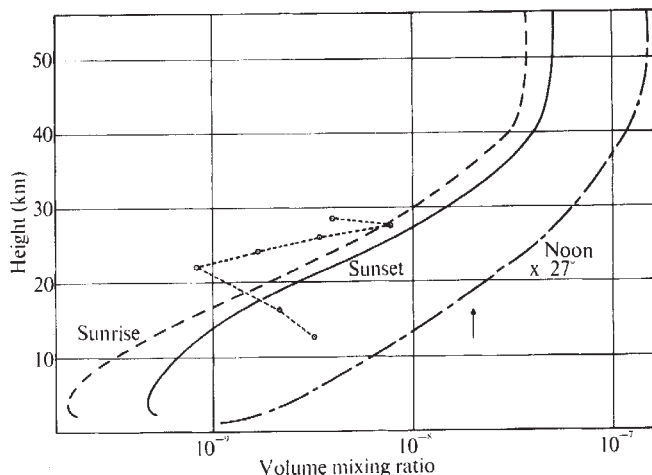


Fig. 5 Vertical distribution of NO_2 in the atmosphere at different times of the day, as measured over Southern Ontario in first half of 1973. For comparison data from Ackerman and Muller⁶ (---) and Harries⁷ (· · ·) are shown on the diagram.

rapidly, and if in this period the NO_2 is not changing too fast then the slope of the line will be the NO_2 present at that time; if extrapolated back to $\sec \chi = 0$, the line will give F_0 , which is the value the function would have if the spectrograph were taken outside the atmosphere. We have normalised this to zero. Thus we consistently obtain a suitable value for the slope and the intersection occurs at a value very much lower than the instrument reads at noon. This indicates that the total NO_2 overhead at noon is very much larger than is overhead at dusk or dawn.

We therefore deduce that the upper air NO_2 has a large diurnal cycle with much larger amounts present when the Sun is high than at dusk or dawn.

Observations at Noon

To investigate this further we made an aircraft flight at local noon with a solar zenith angle of 27° . The aircraft climbed to 11 km and readings were taken of the direct Sun as frequently as possible during the climb and descent. The slope of the observed value of F against height gives the local concentrations. The measured values are shown in Fig. 7. The corresponding NO_2 concentrations are shown in Figs 4 and 5.

If we use these values, together with the total NO_2 , which is obtained from a ground reading at noon and our value of F_0 as discussed above, we have a suggested noontime distribution shown by the appropriate lines in Figs 4 and 5.

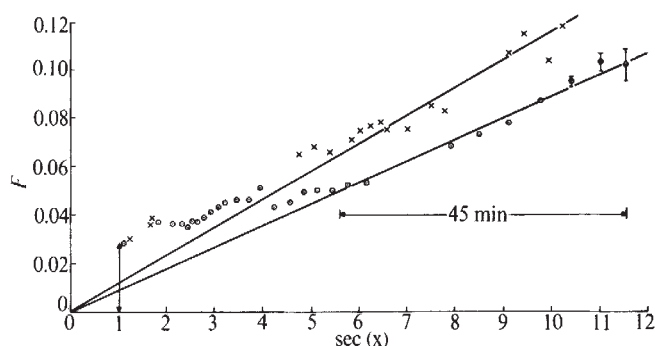


Fig. 6 Observations on the direct Sun, August 3, 1973, Toronto, Ontario. $\times$, Afternoon and evening; $\circ$, morning. The two straight lines are drawn through the points obtained when the Sun is low. Their slope gives the total NO_2 (low level + upper air) at sunset $= 1.65 \times 10^{-3}$ atm cm, and total NO_2 at dawn $= 1.12 \times 10^{-3}$ atm cm. At $\sec \chi = 0$ both extrapolate to F_0 which value is used throughout this work. The total NO_2 at noon was estimated to be 3.5×10^{-3} atm cm.

It is difficult to give absolute limits to the errors possible in these conclusions. The vertical distributions for sunrise and sunset are calculated by finding an NO_2 distribution which, when used in a single scattering model, predicts zenith sky data which agree with observations. The solid lines of Fig. 2a and b are so calculated. If, in any layer between 10 and 40 km, the concentrations are changed from those shown in Fig. 4 by 50% through a depth of one scale height then the fit of the lines to the points becomes unacceptable to the eye. If there were day to day fluctuations of this amount we would notice it in variations of the observations. Similarly if we have wrongly estimated our F_0 by 0.01, which would give an error of 50% in the high noon total of NO_2 , then the curves would be displaced by this amount and it would again be obvious. Errors as large as this therefore seem unlikely.

The diurnal cycle is thus the opposite of that observed in low level NO_2 and is the reverse of that usually hypothesised to occur in the stratosphere. Perhaps the importance of the presence of ozone is underestimated. In the presence of an

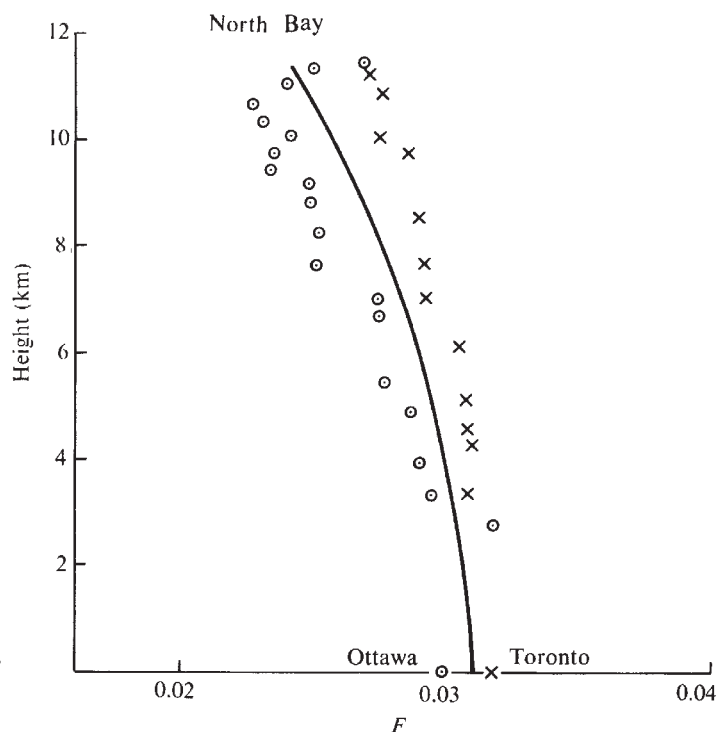


Fig. 7 Direct Sun observations at noon, August 3, 1973. For practical reasons the aircraft flight was from Ottawa climbing to North Bay and descending to Toronto. $\circ$, Observed in ascent; $\times$, observed in descent. Solar zenith angle, 27° .

excess of ozone, as occurs throughout the upper atmosphere, NO_2 reacts with ozone to give NO_3 ($\text{NO}_2 + \text{O}_3 \rightarrow \text{NO}_3 + \text{O}_2$). This reaction, which has been discussed by Johnston⁵, is easy to demonstrate in the laboratory. Ozone and NO_2 will exist together in a glass vessel for days showing the clear blue of NO_3 . We presume that the NO_2 goes to NO_3 by night which is photolysed back into NO_2 by day. NO_3 has optical absorption coefficients (H. Johnston, private communication) which should make its measurement with our apparatus possible. We hope to do so.

Overall Distribution of Atmospheric NO_2

(1) Atmospheric NO_2 occurs in two layers which have very different properties.

(2) Near the ground in relatively unpolluted Canadian air there is a very variable amount of NO_2 of the order of 10^{-3} atm cm. This is present at night. It tends to be larger at dawn than at sunset and is greatly reduced when the Sun is high.

(3) For bright, clear days the total NO_2 overhead in the upper atmosphere does not seem to change very much from day to day, but it has a strong diurnal variation, with a maximum near noon. There is about 0.35×10^{-3} atm cm overhead at sunrise, about 3.0×10^{-3} atm cm when the Sun is high though this measurement is very difficult, and about 0.55×10^{-3} atm cm at sunset.

(4) The concentration of the NO_2 does not change rapidly with height, probably up to 40 km. Expressed as mixing ratio it increases strongly with height probably at least to 40 km. We note that this distribution will result in very heavy downward transport of NO_2 and its associated compounds from the upper stratosphere into the troposphere. Suggested distributions at sunrise, high noon, and sunset are given in Fig. 4.

(5) It seems it would be profitable to look for NO_3 in the atmosphere. We hope to do this.

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Structure of the λ Operators

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The two regions of λ DNA to which the λ repressor binds (the operators) are shown to consist of multiple, non-identical repressor binding sites. The arrangement of these sites with respect to the adjacent controlled genes has been determined using a restriction endonuclease that cleaves each operator.

THE sites on DNA to which repressors bind are called operators. In the DNA of bacteriophage λ , two operators are recognised by the same protein, the λ phage repressor. These operators are separated by about 3,000 base pairs (bp), and they independently regulate transcription of two separate operons. Repressor bound to O_L blocks leftward transcription of gene N , and rightward transcription of gene tof is blocked by repressor bound to O_R (Fig. 1, and for review see ref. 1).

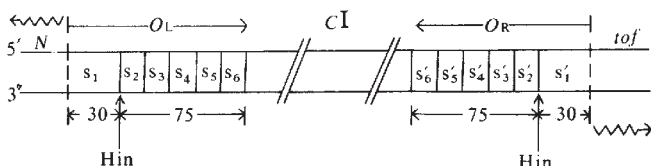


Fig. 1 Schematic representation of the λ operators and adjacent genes. The wavy arrows show the direction of transcription of the repressor-controlled genes N and tof . Two arrows indicate the order of repressor binding from the first sites (s_1 and s'_1) to the final sites (s_6 and s'_6) in O_L and O_R . The Hin cutting sites in O_L and O_R are indicated along with the number of base pairs within the operators on either side of each Hin cut. The repressor gene is cI .

In this paper we describe certain aspects of the structure of the two λ operators. Our conclusions are summarised in Fig. 1, the essential features of which are as follows. Each operator consists of multiple, non-identical repressor-binding sites. These sites are arrayed in the order shown in the figure: at O_L , repressor first binds to a site (labelled s_1), which is about 30 bp long and is adjacent to N ; subsequent repressor molecules add in linear rightward order to the remaining sites

s_2 – s_6 , each of which is about 15 bp long. At O_R the reverse orientation is found: the site to which repressor first binds (s'_1) is at the right terminus, and subsequent repressor molecules bind in linear leftward order. We present here only the essential evidence for these assertions; a more complete description of some of these experiments is found in ref. 2.

The λ operators may be isolated by digesting ^{32}P -labelled λ DNA with DNase in the presence of λ repressor^{3,4}. The protected fragments, bound to repressor, are then trapped on nitrocellulose filters, eluted and examined on polyacrylamide gels. Using the appropriate mutant DNAs it is possible to isolate O_L and O_R separately. We⁴ obtained results of an unanticipated complexity from these nuclease digestion experiments. When the ratio of repressor to operator was increased, six successively larger DNA duplexes were recovered from each operator. The smallest of these was about 35, the largest about 100 bp long, and the intermediate fragments differed in length by about 10 to 15 bp. Analysis of the pyrimidine tracts of these fragments revealed that, at each operator, the site to which repressor first binds is unique (though not necessarily terminal), and that the adjacent sites are then filled sequentially. Within each operator the adjacent sites are not identical repeating sequences and, moreover, the sequences in O_L differ from those in O_R , a finding consistent with the observation that O_L and O_R differ in their affinities for repressor.

Restriction Endonucleases

Our experiments exploit two restriction endonucleases, each of which makes over thirty-five double stranded cuts in λ DNA. One, called Hin , is isolated from *Haemophilus influenzae*, and the other, Hpa , is isolated from *Haemophilus parainfluenzae*. Each is known to be a mixture of more than one restriction enzyme (ref. 5 and Smith, personal communication), but we have found it unnecessary to fractionate these activities. Figure 2 shows the cutting sites of Hpa and Hin in the region of O_L and O_R . The location of these cutting sites was determined in most cases by hybridising the fragments to various deletion and substitution mutants of λ , and in some cases by digesting mutant DNAs and examining the products on polyacrylamide gels². The sizes of the various fragments produced by Hin and/or Hpa , indicated on the figure, were determined as described in ref. 2.

Our restriction endonuclease experiments received their initial impetus from the discovery of Allet⁶ that one of the fragments generated by the action of Hpa on λ DNA is located near the beginning of N , and another includes tof . In collaboration with Allet we found that these fragments, 350 and

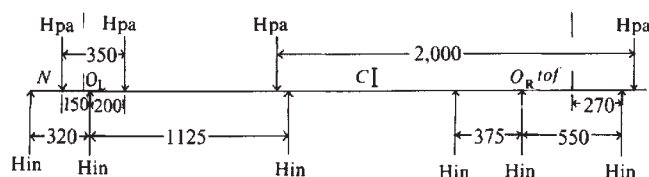


Fig. 2 Schematic representation of a portion of the λ genome showing portions of Hpa and Hin cleavage sites in and around O_L and O_R . The approximate numbers of base pairs between the various cutting sites are indicated. The determination of the various cutting sites is described in ref. 2.

2,000 bp long, bind repressor (unpublished), and further experiments confirmed that they contain intact O_L and O_R respectively². Encouraged by this indication that O_L and O_R can be separated, we sought and found another restriction endonuclease that cuts within each operator. As we shall show, this enzyme (Hin) splits the first repressor binding site from the remaining sites in each operator, and Hin fragments containing either the primary or the remaining operator sites bind repressor.

Operator Fragments produced by Hin and Hpa Cutting of Whole λ DNA

When λ DNA is digested with Hpa, two (and only two) fragments are generated which bind repressor. This is shown by the experiment of Fig. 3a. Hpa-digested, 32 P-labelled

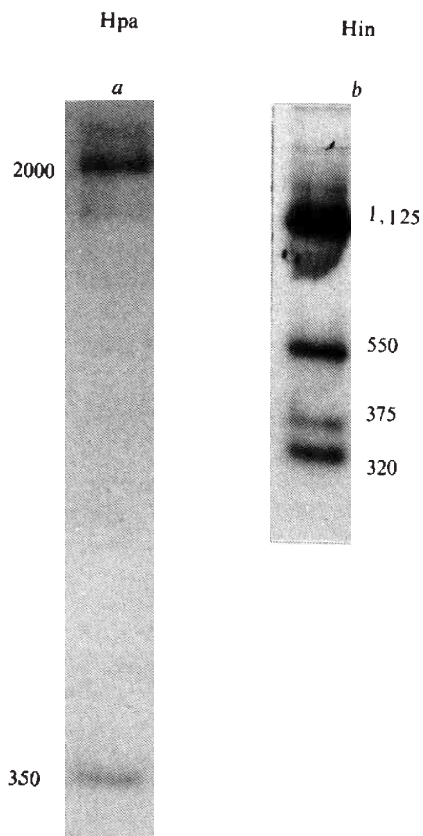


Fig. 3 Operator-containing fragments generated by digestion of whole λ DNA with Hpa and Hin. Hpa and Hin were isolated and used as described in ref. 2. a, 10 μ g 32 P-labelled (1.0×10^6 c.p.m. μ g⁻¹) λ DNA was digested with Hpa for 15 h at 37° C in Hpa buffer². Following phenol extraction, a two-fold excess of λ repressor was added to the digested DNA and the mix passed through a nitrocellulose filter. DNA eluted from the filter^{3,4} was examined by electrophoresis through a 4% polyacrylamide Tris-acetate gel¹². b, 25 μ g (5×10^5 c.p.m. μ g⁻¹) 32 P-labelled λ DNA was digested with Hin for 15 h at 37° C in Hin buffer²; thereafter the samples were treated as in a. Chain lengths of the duplexes eluted from filters are indicated on the figure.

λ DNA was mixed with λ repressor, passed through a nitrocellulose filter, and the fragments trapped on the filter by repressor were eluted and examined by gel electrophoresis. Two fragments, 350 and 2,000 bp long, were recovered. (The total digest contained over fifty specific fragments (see Fig. 4b of ref. 2).) As indicated in Fig. 2, Hpa 350 contains an intact O_L and Hpa 2,000 an intact O_R .

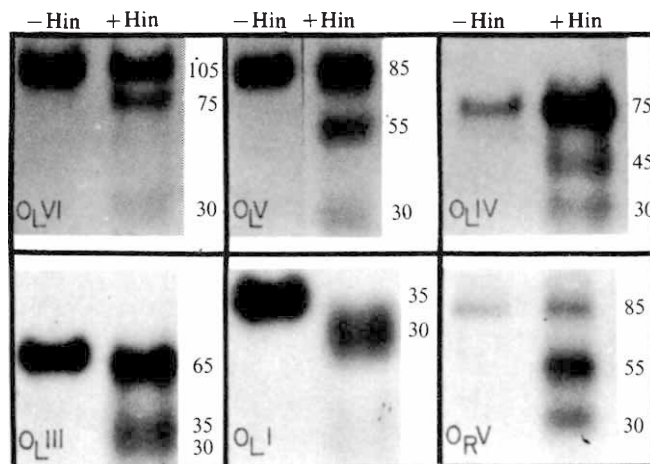


Fig. 4 Hin cutting of isolated operators. Operator fragments (O_{LI} , III, IV, V, VI and O_{RV}) were purified as previously described⁴, digested with Hin for 15 h at 37° C in Hin buffer², and subjected to electrophoresis on a 12% polyacrylamide Tris-Borate-Mg²⁺ gel⁴. Columns labelled '-Hin' show operator fragments incubated under the same conditions but without Hin. Chain lengths are indicated on the figure. In every case except one (O_{LI}) the columns labelled '+Hin' contain some undigested operator, indicating that a limit digest was not achieved.

When this experiment was performed with Hin instead of Hpa, we observed a strikingly different result. In this case four fragments were selectively bound to the filter by λ repressor, indicating that Hin cuts within each operator once, generating two repressor-binding fragments from each operator (Fig. 3b). As shown in Fig. 2, two of these Hin fragments (Hin 320 and Hin 1,125) contain portions of O_L , and the other two (Hin 550 and Hin 375) contain portions of O_R . As predicted from these results, two repressor binding fragments are generated by Hin digestion of either operator-containing Hpa fragment (Fig. 5 of ref. 2). An important conclusion from these experiments is that the secondary repressor binding sites specifically bind repressor even when separated from the primary repressor binding sites in each operator. Thus each operator must contain more than one sequence recognised by repressor. A direct confirmation that Hin cuts each operator is presented below.

Hin Cuts Isolated λ Operators

Five operator fragments were purified from O_L by pancreatic nuclease digestion of the appropriate λ mutant DNA in the presence of varying amounts of repressor as described previously⁴. These duplexes, designated O_{LI} , III, IV, V and VI, are 35, 65, 75, 85 and 105 bp long. Each of these fragments was treated with Hin and the products examined by gel electrophoresis (Fig. 4). In each case two fragments were generated; one was about 30 bp long, and the other varied in length depending on the size of the particular O_L fragment. A schematic representation of the results is presented in Fig. 5. Two important conclusions emerge from this result: (1) Hin cuts once in O_L , near the junction between the first (s_1) and second (s_2) binding sites, and (2) s_1 is terminal, and the remaining sites are filled in linear order.

This experiment has also been performed using the 85 bp operator fragment isolated from O_R (O_{RV}). In this case the

same result was observed as with the corresponding $O_L V$ (Fig. 4). From experiments described below we deduce that the 30 bp fragment split from $O_R V$ by *Hin* is s_1 . We conclude therefore that as in O_L , the first repressor binding site in O_R (s_1) is terminal, the sites are filled in linear order, and *Hin* cuts between s_1 and s_2 .

Orientating the Operators

We have argued that one of the many *Hin* cutting sites in λ DNA is within O_L between s_1 and s_2 , and another is within O_R between s_1' and s_2' . This conclusion predicts that of the two O_L -containing *Hin* fragments generated from whole λ DNA, one should contain s_1 , the other s_2-s_6 . Similarly, the two O_R -containing fragments should contain, separately, s_1' and $s_2'-s_6'$. The experiment of Fig. 6 verifies this prediction and, moreover, reveals the important new fact that each operator is orientated such that s_1 and s_1' are proximal to *N* and *toF* respectively. To perform this experiment, the four operator-containing *Hin* fragments, labelled with ^{32}P , were isolated from whole λ DNA as described for the experiment of Fig. 3, digested with pancreatic DNase in the presence of excess repressor, and recovered from nitrocellulose filters. Figure 6 shows that the two *Hin* fragments known to contain *N* and *toF* sequences (*Hin* 320 and *Hin* 550, respectively, see Fig. 2) yielded duplexes 30 bp long, the known sizes of s_1 and s_1' . Larger pieces were protected from the other two *Hin* fragments: the fragment containing the right part of O_L (*Hin* 1,125) and that containing the left part of O_R (*Hin* 375) yielded, respectively, duplexes 75 and 55 bp long which we identify as s_2-s_6 and $s_2'-s_6'$. (The fact that s_6 was not protected in this particular experiment is consistent with our observation that, compared with O_L , more repressor is required to protect the largest fragment of O_R (ref. 4).)

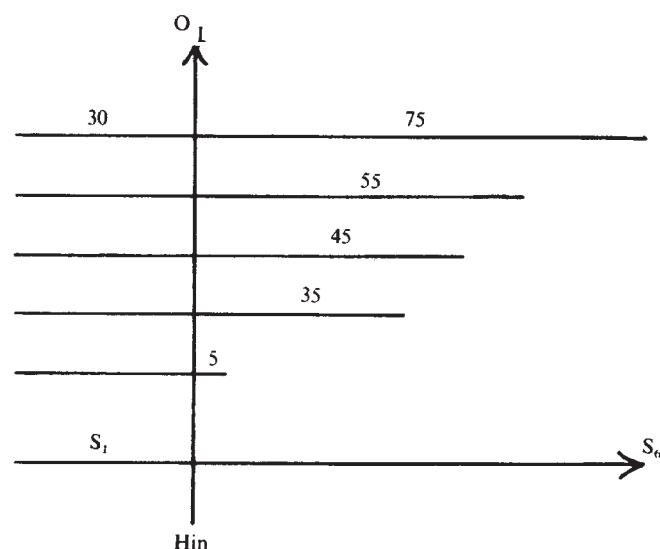


Fig. 5 Schematic representation of the effect of *Hin* on isolated O_L fragments. The vertical arrow labelled *Hin* shows the position of the cutting site within the various operator fragments. The horizontal arrow shows the order of repressor binding sites s_1 to s_6 .

The results of the experiment of Fig. 6, taken in conjunction with the fact that each operator is filled with repressor in linear order, orientates each operator as shown in Fig. 1. s_1 and s_1' are proximal to *N* and *toF* respectively. O_L binds repressor beginning at its left extremity and subsequent repressor molecules are added in a rightward direction, whereas the opposite is true at O_R .

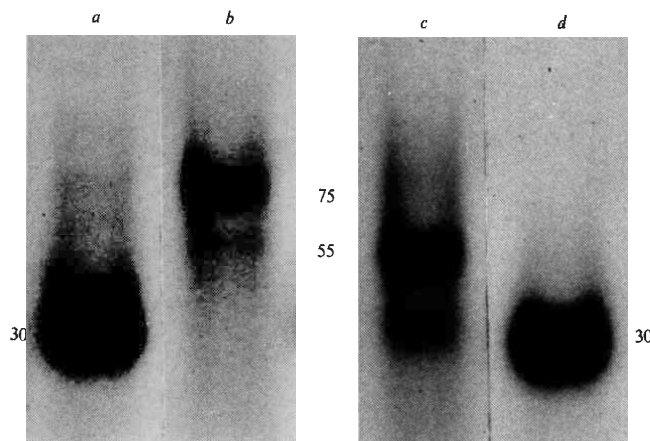


Fig. 6 Operator fragments contained in four *Hin* fragments. The four operator-containing *Hin* fragments were isolated as in Fig. 3b and eluted from the gel. Repressor was added to ten-fold molar excess and the samples digested with DNase^{3,4}. The protected fragments were examined on 12% TBM polyacrylamide gels⁴. Sizes of the protected fragments are indicated on the figure. a, *Hin* 320; b, *Hin* 1,125; c, *Hin* 375; d, *Hin* 550.

Further Results and Discussions

Several additional findings, evidence for which may be found in ref. 2, are as follows: (1) The two *Hin* fragments identified as containing s_1 and s_1' (*Hin* 320 and *Hin* 550) bind repressor more tightly than do the two *Hin* fragments containing s_2-s_6 and $s_2'-s_6'$ (*Hin* 1,125 and *Hin* 375). The order of affinities is *Hin* 320 > *Hin* 550 > *Hin* 1,125 > *Hin* 375. We estimate that, for half-maximal binding, s_2-s_6 requires about three- to five-fold more repressor than does s_1 , and $s_2'-s_6'$ requires about ten- to fifteen-fold more repressor than does s_1 as measured using the various operator-containing *Hin* fragments. (2) An operator constitutive mutation has been located in s_1 . (The repressor affinity of *Hin* 320, but not that of the remaining three operator-containing *Hin* fragments, is reduced (about three- to five-fold) when *Hin* fragments are generated from DNA bearing this mutation.) (3) The distance between the left extremity of s_1 and *N* is less than 20 bp.

The experiments reported here, taken in conjunction with our earlier work², show that each λ operator contains multiple repressor recognition sites. In all, there are at least four and may be as many as twelve different sites specifically recognised by one protein, the λ phage repressor. Although the two λ operators differ in their sequences and affinities for repressor⁴, the arrangement of binding sites with respect to the adjacent controlled genes is the same in the two cases. In both cases the strongest affinity site is adjacent to the controlled gene, and repressor fills the operator linearly in the direction opposite to that of transcription of the structural gene. At least one sequence is common to both operators, namely, the *Hin* cutting site located between the first and second binding sites in each operator.

The λ operators are more complex than the *lac* operator, which consists of a single sequence about 25 bp long⁷. It may be relevant to note that the only molecular species of *lac* repressor known to bind DNA is a stable tetramer of identical subunits of molecular weight 40,000 (refs 8, 9). The λ repressor, in contrast, has the property that its subunit (molecular weight 30,000) is in concentration-dependent equilibrium with dimers and tetramers^{10,11}, and we deduce from the different sizes of the binding sites on λ DNA that more than one form of the λ repressor binds to DNA. Whatever the significance of these observations may be, one principle of DNA-protein interaction that emerges from the study of the λ case is that the presence of multiple, non-identical binding sites enables a small protein to cover a relatively long and unique stretch of native⁴ DNA.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Deuterium Formation Hypothesis for the Diffuse Gamma-ray Excess at 1 MeV

MANY measurements of the diffuse X-ray and γ -ray background¹⁻⁴ indicate that it consists of an inverse power law spectrum with index ~ 2.0 . Spectral features or excesses are reported in the vicinity of ~ 40 keV and ~ 1 MeV. When plotted on linear-linear paper the 1 MeV excess is seen to consist of a pronounced peak in the 0.5 to 2.0 MeV region with a feeble tail extending on to higher energy (Fig. 1). In this paper a simple explanation is presented for the 1 MeV peak in light of new experimental and theoretical advances made in the past year. No explanation is presented for the higher energy excess and it is presumed to come from other mechanisms. I hypothesise that the peak arises from the astrophysical production of deuterium by the two body recombination process ($p+n \rightarrow D+2.2$ MeV γ ray) in an environment transparent to the emitted γ rays. The hypothesis is shown to lead unambiguously to two remarkable numerical results: a calculation of the terrestrial $n(D)/n(H)$ ratio; and a prediction of QSO cutoffs at $z \sim 3$.

The most recent measurement of the γ -ray background made on Apollo 15 has confirmed the 1 MeV feature⁴. Although the large uncertainties associated with this spectrum ($\sim 30\%$) may seriously effect the shape and magnitude of any extracted excess curve, it nevertheless seems reasonable to accept the data at face value and investigate the consequences, bearing in mind that future, more precise data may change the arguments considerably. In Fig. 1 the difference between the Apollo 15 spectrum and an $E^{-2.0}$ extrapolation of the low energy power law portion of the Apollo 15 spectrum is plotted. (The discussion to follow is substantially unaltered by changes in the power of the extrapolation $\lesssim 10\%$.) The Apollo 15 spectrum has been used because it is most recent and spans both the power law and 1 MeV excess regions of the spectrum. Several origins of the feature have already been suggested. These are summarised and discussed in a recent paper by Stecker³. At present none of these has yet been widely accepted. Here I argue generally in support of the position of Clayton and Silk⁵ who suggest that the origin of the feature is in γ -ray lines emitted by heavy radioactive nuclei after their

synthesis in supernova explosions throughout the Universe. But it is suggested in light of recent discoveries that Clayton and Silk⁵ (CS) have not discussed what may be the most important γ -ray line emitted in astrophysical nucleosynthesis, namely the 2.2 MeV deuterium recombination line. The large potential enhancement in γ -ray line signal from this mechanism significantly strengthens the argument for a line origin of the feature.

The unambiguous astronomical detection of deuterium^{6,7} has now removed all doubt concerning its existence on a galactic scale. Some doubt exists concerning its relative abundance, but it is reasonable to assume that the $n(D)/n(H)$ ratio approximates the terrestrial value of $1-2 \times 10^{-4}$ on a galactic scale. In view of this large abundance, Hoyle and Fowler⁸ and Colgate⁹ have argued that the D may be made astrophysically under non-thermodynamic conditions rather

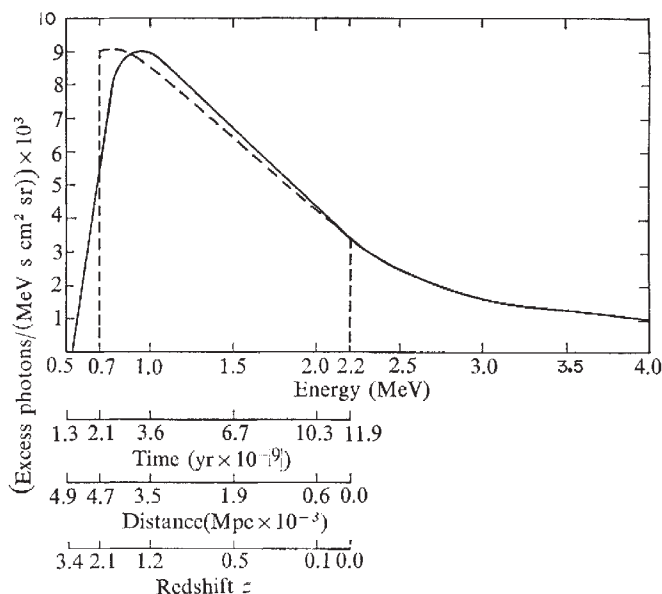


Fig. 1 Plot of the 1 MeV diffuse γ -ray excess above an $E^{-2.0}$ power law extrapolation of the lower energy spectrum. Also shown (---) is a theoretical spectrum based on the deuterium formation hypothesis and cosmological assumption discussed in the text. The energy axis is also labelled in time and distance for the cosmological model used.

than cosmologically. Specifically Hoyle and Fowler suggest that α particles accelerated in a supernova shock wave can produce D and neutrons in the outer envelope by a spallation reaction and the neutrons may subsequently recombine with the ambient hydrogen also to make D. In view of the present rudimentary understanding of the details of supernova explosions, it is difficult to decide whether the direct production of D is more important than the indirect recombination process. Hoyle and Fowler⁸ conclude that the recombination process can be an order of magnitude more efficient if the H gas density is high enough for most of the neutrons to be captured.

Strong evidence in support of the contention that recombination can be an important astrophysical process in an environment which is transparent to 2.2 MeV γ rays was obtained by Chupp *et al.*¹⁰. Observation of two intense solar flares with a satellite borne detector revealed that the dominant feature in the γ -ray spectrum was the 2.2 MeV deuterium recombination line. Ramaty and Lingenfelter¹¹ have shown that the propagation of ~ 10 MeV neutrons in an H atmosphere is determined by elastic and inelastic np scattering with D formation occurring at a range of about 1.6 g cm^{-2} . Since the stopping range of a 2.2 MeV photon is about 25 g cm^{-2} these workers conclude that the solar flare γ rays will escape unattenuated. Although I am not arguing for a solar flare origin of the 1 MeV excess, it is important to see that it is clearly possible to construct astrophysical models which will be compatible with the D hypothesis.

On the basis of a simple abundance argument, $(n(^{56}\text{Fe})/n(\text{H})) = 3.1 \times 10^{-5}$) and the assumption that the ^{56}Fe in the Universe was produced by radioactive decay from supernova produced ^{56}Ni and ^{56}Co , CS concluded that the dominant lines in the 1 MeV excess should be from this process. Their flux estimate indicated that it might be possible to account for most of the excess with these lines. Assuming that recombination is the important D production mechanism on a cosmic scale and noting that the $n(\text{D})/n(\text{H})$ ratio may be an order of magnitude greater, I conclude that D recombination can more easily account for the excess. The subsequent discussion does not depend on a supernova origin of D but only on the pre-eminence of astrophysical D production by recombination and the transparency of the source to 2.2 MeV γ rays. But D formation may take place in the outer envelope of supernova from which the probability of γ -ray detection could be high relative to γ -ray detection of heavy element nucleosynthesis at the core which must await the expansion of the remnant before becoming detectable. Once out of the source, absorption in the interstellar and intergalactic medium is expected to be negligible.

The exact shape of the expected D feature depends on the cosmological model one assumes and also on assumptions concerning the time dependence $f(t)$ of the galactic synthesis rate of D. Clayton and Silk have derived expressions for the shape of the spectral feature anticipated from a single γ -ray line. Clearly one expects an edge at the rest energy of 2.2 MeV and a continuous distribution towards redshifted energies from D synthesised at earlier times in distant galaxies. Eventually at lower energies the distribution will disappear as one looks at the epoch prior to the beginning of astrophysical nucleosynthesis assumed to start at time t^* in all the galaxies.

To show that the observed feature is consistent with reasonable cosmological assumptions a computer constructed plot of the expected shape is given in Fig. 1 for an Einstein-de Sitter universe, $q_0 = 0.5$, $\rho_0 = 5 \times 10^{-30} \text{ g cm}^{-3}$ for which $\gamma = 1.5$. Employing the current value of $55 \text{ km s}^{-1} \text{ Mpc}^{-1}$ for the Hubble constant yields a universe of age $t_0 = 11.9 \times 10^9 \text{ yr}$. The data indicate a rate of nucleosynthesis peaked in the past for which an exponential form $f(t) = f_0 \exp[-\lambda t]$ has been assumed. Following CS, equation (8), a theoretical flux curve of the form

$$F \propto \exp(-\lambda t_0(E/2.2)^\gamma)(E/2.2)^{\gamma-1} \text{ cm}^{-2} \text{ s}^{-1} \text{ sr}^{-1} \text{ MeV}^{-1}$$

amplitude normalised to fit the data at 2.2 MeV has been used.

At this point it is important to point out that t_* , λ , and f_0 are now determined by the data. The low energy edge at E_* determines $t_* \sim 2.1 \times 10^9 \text{ yr}$ through the expression $t_* = t_0(E_*/2.2)^\gamma$; the shape of the 1 MeV feature determines $\lambda^{-1} \sim 6.5 \times 10^9 \text{ yr}$; and its amplitude determines f_0 . The quantity f_0 can most easily be determined from a cosmological correspondence principle—for small distances and redshifts the results of relativistic calculations in curved spaces must go over to the results of non-relativistic calculations in Euclidean spaces. So the incremental flux ΔF in the energy interval E to $E + \Delta E$ and the time interval t to $t + \Delta t$ arises from galaxies at distances r to $r + \Delta r$ and is given non-relativistically for small r by

$$\Delta F = (1/4)n_g f_0 \exp(-\lambda t) \Delta r \text{ cm}^{-2} \text{ s}^{-1}$$

where n_g is the average density of galaxies taken to be $1.0 \times 10^{-75} \text{ cm}^{-3}$. The factor $1/4$ arises $(1/2)$ from the fact that γ rays emitted on the far side of a thick synthesising object like a supernova have no chance of reaching us and $(1/2)$ from the angular distribution of galaxies with respect to a flat 1-cm^2 detector. Using the value of λ determined from the shape of the excess and evaluating ΔF for an interval Δr near $r=0$ yields $f_0 \sim 1.2 \times 10^{47} \text{ s}^{-1}$. In the above estimate I have neglected: (1) all other possible contributions to the 1 MeV excess (such as the ^{56}Ni and ^{56}Co γ -ray lines; (2) the possibility that nucleosynthesis did not begin at the same time t_* in all galaxies; (3) any Compton scattering of the γ rays before detection; and (4) the large energy resolution ($\sim 10\%$) of the detectors employed on Apollo 15. The sharp edge predicted by the D hypothesis at 2.2 MeV is only hinted at in the data, presumably because of instrumental smearing and background effects.

If the above discussion is taken seriously and absorption of the γ rays in the source is neglected the $n(\text{D})/n(\text{H})$ ratio can be estimated in the Galaxy for D produced by recombination. The total number of D nuclei, $N(\text{D})$, is given by

$$N(\text{D}) = \int_{t_*}^{t_0} f_0 \exp(-\lambda t) dt = 1.3 \times 10^{64}$$

Taking the Galactic mass to be $1.1 \times 10^{11} M_\odot$ containing 70% H by mass yields $N(\text{H}) = 9.2 \times 10^{67}$. Thus a D/H ratio of $\sim 1.5 \times 10^{-4}$ is obtained which is in agreement with the terrestrial value. Moreover this result is relatively insensitive to cosmological models which basically serve to fix the time axis of Fig. 1 and the specific form of $f(t)$, depending primarily on the area under the curve in Fig. 1. But in the light of the many uncertainties in the above estimate the exact agreement with the terrestrial value must be fortuitous, although it nevertheless indicates that the calculation gives an order of magnitude estimate which is correct. I take this remarkable result to be significant evidence for the correctness of the deuterium hypothesis and hope that it will stimulate the search for the astrophysical origin of D. In particular, models of supernova which allow for large scale D production by recombination and the detection of the 2.2 MeV γ rays should be pursued.

Additional support for the D hypothesis comes from the observation of quasar redshifts $z(z \equiv 2.2/E - 1)$. If QSOs are indeed young galaxies and it is assumed that their emission is not occurring before the beginning of D nucleosynthesis at time t_* then Fig. 1 predicts the large z redshift behaviour to be expected. It is apparent that the number of quasars detected at $z \sim 2$ should be falling off rapidly with the distribution disappearing at $z \sim 3$. This agrees with the experimental evidence.

Finally, experimental support for the deuterium hypothesis could come with the detection of a strong 2.2 MeV line from a reasonably local supernova explosion and/or the detection of a 2.2 MeV edge in the diffuse background. For these measurements the application of a high resolution γ -ray telescope is indicated. Detection of large amounts of D in

the vicinity of a supernova remnant by other means would also be supporting evidence.

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Average Trace Element Composition of Low Level Marine Atmospheric Particulates

GROWING concern has been expressed^{1,2} over the introduction of pollutant trace metals to the oceans through the atmospheric transport of particulates from the continents. Few data exist on the elemental composition of such particulates, and those available are usually from restricted regions³⁻⁵. We have carried out a sampling programme^{6,7} in which particulates have been collected from seawater and from the lower atmosphere (~15 m above the sea surface) over large tracks of the world ocean. The collection details have been described elsewhere⁶ and the distribution of Pb in Atlantic particulates has been reported⁷. Here we present data on the average trace metal composition of aeolian particulates from the lower atmosphere of various major wind systems over the World Ocean.

Particulates were sampled from the following wind systems (number of samples in parentheses): North Atlantic Westerlies (4); Atlantic Westerlies/North East Trades boundary region (3); Atlantic North East Trades (7); Atlantic ITCZ (3); Atlantic South East Trades (5); variable winds, South African Coast (7); North-east Monsoon, northern Indian Ocean (3); variable winds, southern Indian Ocean (3); South-west Monsoon, South China Sea (9); northerly wind, East China Sea (1); variable winds, Japanese coast (6). The average trace metal composition of each of these dusts is given in Table 1.

The distribution of some trace elements in low level marine aeolian dusts can vary geographically⁷. This is particularly true of those elements which have a significant man-induced (anthropogenic) component because the relative proportion of this component will decrease with distance from a specific source of pollution. As a result, concentration ranges in the dusts can be relatively large. But in particulates which are not obviously associated with known sources of pollution it is difficult to distinguish between a natural and an anthropogenic origin of trace elements unless there are data on the average distribution of such elements in aeolian particulates from the

low level marine atmosphere. The data given in Table 1 will help to provide such an average.

To identify those trace elements which potentially have a significant anthropogenic contribution Goldberg² has employed an 'enrichment factor', based on the ratio of an element to iron in atmospheric particulates, divided by the ratio of the same elements in average crustal material. Similar calculations have been made for the elements in the particulates from the low level marine atmosphere. Since the ultimate source of natural trace elements in atmospheric particulates is crustal material, from which they are released by the action of geological processes, the enrichment factors should be around unity if natural sources predominate. But these trace elements can undergo considerable fractionation during processes such as weathering and degassing, and the enrichment factors can only be regarded as indicating to a first approximation those elements which potentially have a significant contribution from an anthropogenic source. From the results which are given in Table 1 it can be seen that for the elements Mn, Ni, Co, Ga, Cr, V, Ba and Sr the enrichment factors are reasonably close to unity; in other words, these elements are not significantly enriched in average low level marine aeolian particulates, and in fact Co and Sr are depleted, relative to average crustal material. In contrast, the elements Sn, Pb and Zn are enriched in the aeolian particulates, relative to average crustal material, by one order of magnitude even when geographical variations are smoothed out by taking a 'world-wide' average. These factors have been used to identify those elements which are contaminating the atmosphere², and it may be concluded that Sn, Pb and Zn are being released preferentially into the marine atmosphere (from sources which probably include the activities of man) and are concentrating, relative to average crustal material, in low level aeolian particulates.

Table 1 Average Trace Element Composition of Low Level Marine Atmospheric Particulates

Element	Concentration	Enrichment factor *
Fe	5.2 (2.2-9.9)	—
Sr	101 (17-203)	0.35
Co	9 (3-36)	0.45
Cr	85 (21-850)	1.2
V	145 (37-530)	1.4
Ba	487 (57-1,100)	1.4
Mn	1,312 (310-3,720)	1.5
Ni	91 (10-720)	1.5
Ga	21 (5-41)	1.6
Cu	157 (23-490)	3.4
Zn	683 (136-3,100)	13
Sn	30 (1-120)	19
Pb	465 (29-2,750)	49

* See text.

Concentrations are in p.p.m., with the exception of Fe which is given in wt %; the figures in parentheses indicate the ranges found.

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New Radiolarian Palaeoclimatic Index in the Plio-Pleistocene of the Southern Ocean

PLIOCENE-PLEISTOCENE palaeoclimatic investigations in the Southern Ocean have been carried out using five groups of microfossils: foraminifera^{1,2}, calcareous nannofossils³, Radiolaria⁴⁻⁹, diatoms¹⁰, and silicoflagellates¹¹. Radiolarian methods are particularly valuable in higher latitudes where carbonate sediments are susceptible to dissolution even in relatively shallow waters^{12,13}. Established radiolarian techniques are based on oscillations of cold-water (Antarctic) relative to warmer-water radiolarian assemblages, established by determining their distribution in Recent sediments relative to the Antarctic Convergence (Polar Front)⁴. The cold-water assemblage used in these palaeotemperature determinations consists of eight species, whereas the warm-water assemblage consists of nine species^{4,9}. Of the several dozen species known in Southern Ocean Quaternary sediments, these seventeen species represent more than 75% of the individuals⁴.

Here I describe a new radiolarian palaeoclimatic method, based on frequency oscillations of *Antarctissa strelkovi* Petrushevskaya, which has been applied to Antarctic and Subantarctic deep sea cores. This species comprises a significant portion of the radiolarian assemblage throughout the Pliocene-Pleistocene in Antarctic-Subantarctic cores.

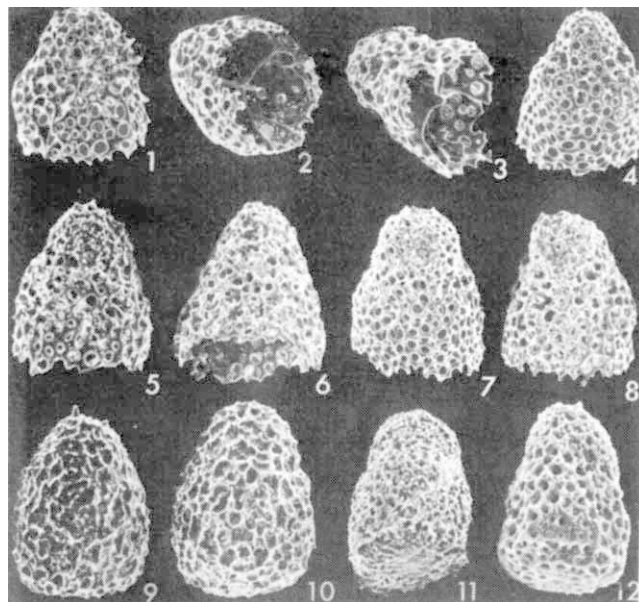


Fig. 2 Nos 1 to 8 *Antarctissa strelkovi* Petrushevskaya: 1, E15-16, 920 to 923 cm, $\times 116$; 2, internal structure, E36-26, 1,222 to 1,224 cm, $\times 107$; 3, internal structure, E36-26, 1,222 to 1,224 cm, $\times 108$; 4, E15-16, 920 to 923 cm, $\times 76$; 5, E21-17, 169 to 171 cm, $\times 92$; 6, E15-16, 920 to 923 cm, $\times 108$; 7, E15-16, 597 to 600 cm, $\times 86$; 8, E21-17, 169 to 171 cm, $\times 88$. Nos. 9 to 12, *Antarctissa denticulata* Petrushevskaya: 9, E36-26, 1,222 to 1,224 cm, $\times 96$; 10, E36-26, 1,222 to 1,224 cm, $\times 84$; 11, showing thoracic plug, E21-17, 169 to 171 cm, $\times 92$; 12, showing thoracic plug, E35-6, 201 to 203 cm, $\times 106$.

not support the palaeomagnetic data, the discrepancy being attributed to the low magnetic intensity in these cores. Thus, the palaeomagnetic stratigraphy for these two cores was inferred from the biostratigraphy¹.

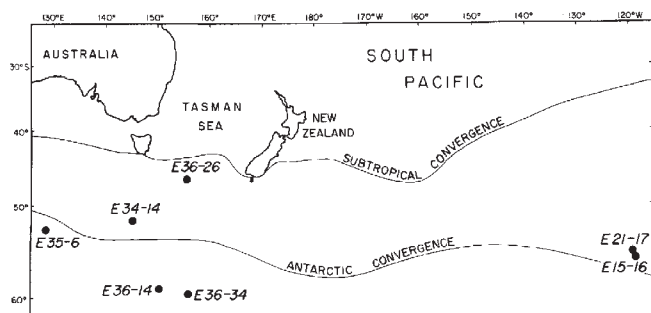


Fig. 1 Map showing location of *Eltanin* cores used in this study and the position of the Antarctic and Subtropical Convergences.

Of the seven cores selected for this study (Fig. 1, Table 1), five are from waters south of Australia (Antarctic waters: E35-6, E36-14, E36-34; Subantarctic waters: E34-14, E36-26) and two are from the central South Pacific (Subantarctic waters: E15-16, E21-17). Four of the five cores south of Australia contain abundant foraminiferal and radiolarian faunas and have palaeoclimatic curves previously established on changes in foraminiferal assemblages². The fifth core, E36-26, has not been previously studied and was selected primarily because it is carbonate free and contains an abundant radiolarian fauna. Subantarctic cores E15-16 and E21-17 have both radiolarian⁹ and foraminiferal¹ palaeoclimatic curves. Two of the seven cores are of Late Pliocene age, and the other five are of Pleistocene age. Palaeomagnetic stratigraphy is available for all the cores^{2,14,15}. For E15-16 and E21-17 the biostratigraphy did

Table 1 Core Parameters

Core	Latitude	Longitude	Water depth (m)	Core length (cm)
E36-26	47° 51' S	155° 05' E	4,691	1,224
E34-14	52° 11' S	145° 03' E	3,264	519
E35-6	53° 12' S	128° 06' E	3,996	600
E21-17	55° 28' S	119° 56' W	2,802	1,004
E15-16	56° 03' S	119° 55' W	3,039	1,200
E36-14	58° 06' S	150° 10' E	3,054	601
E36-34	59° 59' S	155° 02' E	2,794	452

Standard laboratory procedures were followed in the processing of samples and in making faunal slides. Entire slide populations (300 to 1,000 specimens in the $>63 \mu\text{m}$ fraction) were counted for all samples, which were taken at 25 to 40 cm intervals throughout the cores.

Initially, frequency oscillations of *Antarctissa strelkovi* and *Antarctissa denticulata* were investigated. Both forms are consistently abundant throughout the cores. In surface sediments, *A. strelkovi* has its highest frequencies (5% to $>25\%$) in sediments south of 60° S (refs 16 and 17). Petrushevskaya¹⁷ referred to *A. strelkovi* as a high latitude Antarctic species and reported that the distribution of its skeletons in surface sediments of the Southern Ocean corresponds closely with its presence in Antarctic waters. Hays⁴ stated that *A. strelkovi* is probably the most abundant and ubiquitous radiolarian species in the Antarctic, and is the most characteristic species of this fauna. *Antarctissa strelkovi* has also been shown to range from at least the middle of the Gilbert Reversed Epoch to the Recent⁶ ($t=4.0$ m.y. BP to present day). *Antarctissa denticulata* is morphologically similar to *A. strelkovi* and was also

investigated to establish its possible potential as a palaeoclimatic index.

The genus *Antarctissa* is characterised by a compact test, consisting of two segments, the first (cephalis) being somewhat smaller than the second (thorax). The wall of the test has numerous rounded pores, and the surface of the test is generally rough. The internal skeleton consists of

the basic elements typical for nassellarians. *Antarctissa strelkovi* (Fig. 2, Nos 1 to 8) has comparatively thin walls, and the elements of the internal skeleton are more slender and delicate than in *A. denticulata*^{16,17} (Fig. 2, Nos 9 to 12). The massive test of *A. denticulata* has very thick walls, and the thorax is generally closed below by a thick porous plate. The taxonomy and recent distribution of *A. strelkovi*

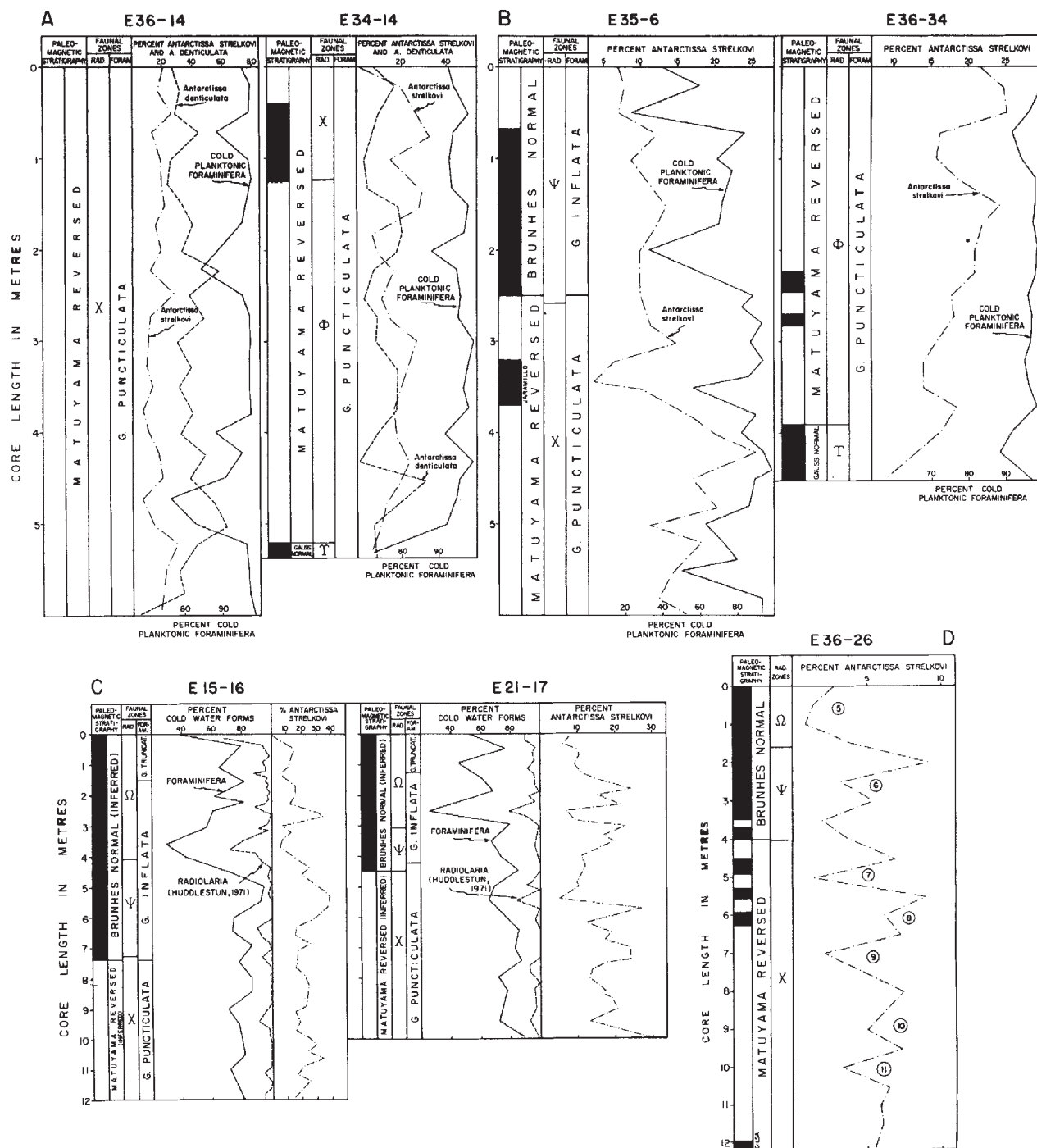


Fig. 3 Comparison of frequency curves of the radiolarian palaeoclimatic index, *Antarctissa strelkovi* with inferred palaeotemperature oscillations in planktonic foraminiferal and radiolarian assemblages in Southern Ocean deep sea cores. A, Comparison of *Antarctissa strelkovi* and *A. denticulata* frequency oscillations with planktonic foraminiferal palaeoclimatic curves², established in two cores of Late Pliocene–Early Pleistocene age south of Australia; B, comparison of *A. strelkovi* frequency oscillations with planktonic foraminiferal palaeoclimatic curves² in two northern Antarctic cores of Late Pliocene–Pleistocene age from south of Australia; C, comparison of *A. strelkovi* frequency oscillations with inferred palaeoclimatic curves based on planktonic foraminifera¹ and Radiolaria⁹ in two Middle to Late Pleistocene cores from the Subantarctic South Pacific; D, frequency oscillations of *A. strelkovi* in a carbonate-free, Early to Middle Pleistocene Subantarctic core. Warm intervals are numbered in accordance with Keany and Kennett². Palaeomagnetic stratigraphy and assigned ages originally given by Watkins and Kennett¹⁴, Kennett¹ and later utilised by Keany and Kennett². Foraminiferal zones after Kennett¹ and Keany and Kennett²; Radiolaria zones (shown by Greek letters) originally defined for the Antarctic area by Hays⁴ and applied to these cores by Kennett¹, Keany and Kennett² and Huddleston⁹.

and *A. denticulata* are discussed in detail by Petrushevskaya^{16,17}.

My first faunal counts were made on E34-14 and E36-14, differentiating *A. strelkovi*, *A. denticulata* and other species. Although the *A. denticulata* oscillations (Fig. 3A) show no consistent similarity to the foraminiferal palaeoclimatic curve, the curves representing the percentages of *A. strelkovi* are very similar to those palaeoclimatic curves; the colder the temperature, the greater the percentage of *A. strelkovi*. There is a good correlation (Fig. 3B) between the number and amplitude of warm-water peaks indicated by foraminifera and those described in this paper. Good correlation can also be observed in the two central South Pacific cores (Fig. 3C). In these two cores the similarities between the two curves established using Radiolaria are greater than those between the radiolarian and foraminiferal curves, suggesting slightly different ecological responses for Radiolaria and foraminifera. The potential value of the *A. strelkovi* method is emphasised by its oscillations in a carbonate free core, E36-26 (Fig. 3D), which contains sediments of Early Pleistocene age (zone X to zone $\Omega^{4,6}$). The palaeoclimatic curve determined for this core is consistent with the palaeomagnetically dated palaeoclimatic curve determined for the Southern Ocean using foraminifera².

In cores of Brunhes and Matuyama age, the curves established using fluctuations in *A. strelkovi* support the conclusions of Kennett¹ and Keany and Kennett² that the Matuyama was, in general, cooler than the Brunhes in Subantarctic and northern Antarctic areas. In the four cores, the percentage of assemblages represented by *A. strelkovi* on average decreases by 26% from the Matuyama Reversed Epoch ($t=2.43$ to 0.69 m.y. BP) to the Brunhes Normal Epoch ($t=0.69$ m.y. BP to present day). In the three cores which contain carbonate sediments of Brunhes and Matuyama age, the boundary between the *Globorotalia inflata* and the *Globorotalia puncticulata* zones¹ occurs within 50 cm above the X- Ψ boundary^{4,6}, therefore coinciding approximately with the Brunhes-Matuyama boundary. Thus, as suggested by Kennett¹, the *G. inflata*-*G. puncticulata* zonal boundary can be used to approximate the Brunhes-Matuyama boundary in carbonate rich cores from the Southern Ocean.

Palaeoclimatic determinations in Southern Ocean cores using the *A. strelkovi* method are limited to the south by the relatively biogenically barren nature of the cores from the Antarctic shelf and to the north by the northern limit of *A. strelkovi*. Cores from the highest latitudes of the Southern Ocean often produce samples containing fifty or fewer Radiolaria, making it difficult to apply radiolarian palaeoclimatic techniques. The scarcity of the radiolarian tests is due to solution of the siliceous tests, dilution by glacial marine sediments and low productivity. In the northernmost core examined (E36-26, 47° 51' S) *A. strelkovi* is less abundant (1 to 9%), and this core probably lies close to the northern limit of the species.

Obvious anomalies in the data occur in two cores. In Antarctic core E35-6, a warm peak at 200 cm suggested by the foraminifera is not as clear in the *A. strelkovi* curve. In Antarctic core E36-14, two very cold episodes indicated by foraminifera at 250 to 375 cm and 600 cm are not reflected by oscillations in *A. strelkovi*.

Determination of oscillations in the frequency of *A. strelkovi* seems to be a valid and rapid approach for palaeoclimatic determinations in the Southern Ocean. Its value is particularly enhanced by the long range of the species from the Pliocene to the Recent and because many Southern Ocean cores are devoid of foraminifera, especially those of Pliocene age.

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Iceland Mantle Plume

O'HARA¹ has objected to my interpretation concerning the trace element chemistry of lavas erupted along the Iceland-Reykjanes Ridge System² on the ground that: (1) Such magmas are not "primary magmas" but residual liquids; (2) instead, such lavas have suffered prior to eruption extensive "gabbro fractionation" at low pressure (olivine-augite-plagioclase extraction), and/or "eclogite fractionation" at higher pressure (clinopyroxene-garnet extraction in a 50-50% mixture); (3) the "extent of fractionation" is directly proportional to the elevation of the surface on which these lavas have been erupted, thus increases toward Iceland, and is the principal cause of trace element variations along the ridge. According to O'Hara, the lavas are derived from a unique and homogeneous mantle at the upper mantle-transition zone boundary and not, as I prefer³, from two distinct mantle sources, one forcefully rising as a primary hot mantle plume (PHMP) beneath Iceland and interacting with the depleted low velocity layer (DLVL).

I answer each of these points in order, concentrating primarily on the rare earth (RE) data which I emphasized², and omitting petrological and isotopic arguments, which will be discussed elsewhere.

Also, several of O'Hara's comments concerning rare earth fractionation are, unfortunately, wrong. For example: (i) Fractional crystallization of clinopyroxene does not deplete but rather enriches a residual melt in lighter rare earths, as is evident from equilibrium partition coefficient data⁴⁻⁶. (ii) There is no relative Y enrichment towards Iceland, because I did not report nor analyse this element, and my expectation is that it will remain relatively constant, similarly to the heavy rare earths of similar ionic size, such as Er. (iii) There is no massive relative and even absolute depletion of Yb as stated by O'Hara to support his eclogite-fractionation model. The Yb abundance remains relatively constant along the ridge up to Iceland (Fig. 1a). Much of the Yb scatter is in fact analytical, as with the

instrumental neutron activation method used the counting statistics are relatively poor for Yb ($\sigma_{Yb}=15\%$, $\sigma_{La}=9\%$, and $\sigma_{Sm}=2.5\%$). (iv) O'Hara's use of the $[La/Yb]_{EF}$ is misleading since it bypasses the behaviour of intermediate rare earths. This ratio does not adequately contrast the behaviour of the light rare earths relative to the heavy rare earth subgrouping. All the information contained in the rare earth pattern needs to be included to constrain the models.

(1) "Primary magmas". This is a semantics problem. I have

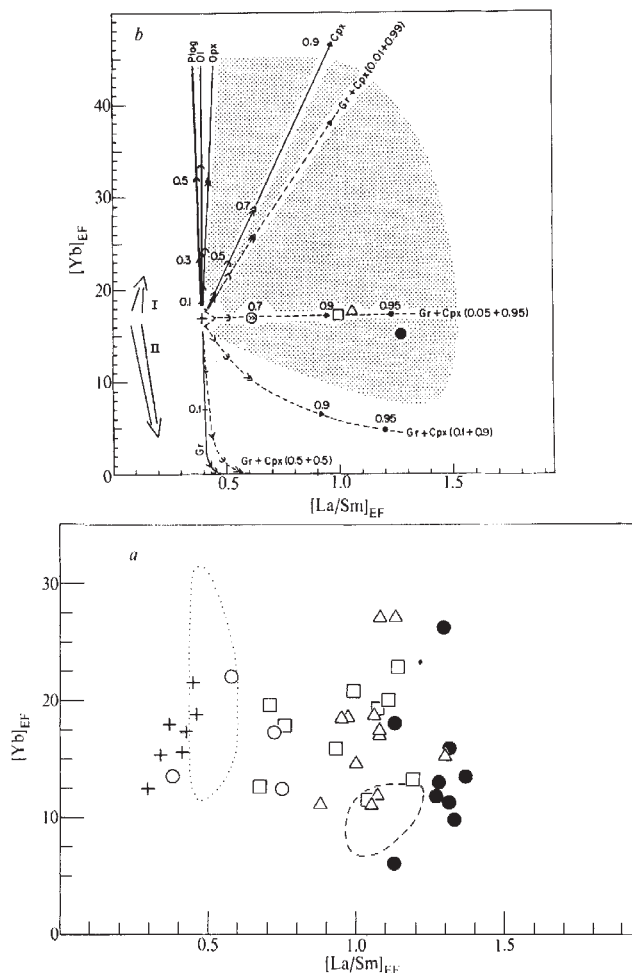


Fig. 1 *a*, Yb against La/Sm enrichment factors relative to chondritic meteorites² in tholeiitic basalts erupted along the postglacial Reykjanes Ridge Axis and its extension over the Middle Neovolcanic Zone of Iceland². +, South of 61°N; ○, 61-62°N; □, 62-63°N; △, 63°N to Iceland; ●, Iceland. Dotted area is field of DLVL-derived tholeiitic basalts from other Mid-Ocean Ridges⁸; and dashed area is field for Hawaiian tholeiites⁹. *b*, Same diagram as above, showing theoretical control liquid lines of descent for fractional crystallization of plagioclase feldspar (Plag), orthopyroxene (Opx), olivine (Ol), clinopyroxene (Cpx) and garnet (Gr). The bar, arrow, half circle, double arrow, filled triangle and point along liquid control lines represent residual liquid composition after fractional crystallisation of respectively 10, 30, 50, 70, 90 and 95 weight % of the corresponding mineral (or biminerals mixtures for eclogite fractionation—shown as dashed liquid control lines). The large symbols are as given in *a* for the observed values in basalts erupted along the Reykjanes Ridge, but averaged for every degree of latitude. Large arrows: I, the approximate direction of olivine-gabbro fractionation; II, of eclogite fractionation. The lengths are very roughly proportional to the rate of enrichment or depletion for a given degree of fractional crystallisation. Various paths to bridge DLVL-derived melts (+) to PHMP-derived melts (●) can be designed. All these paths require a very unusually large extent of fractional crystallisation of clinopyroxene, and cannot at the same time satisfy the major element chemistry of these two end-member tholeiites (which relative to trace elements represent only small variations). The shaded area takes into account the known range of partition coefficients^{4,8} for plagioclase, and the dotted area for the clinopyroxene.

used the adjective "primary" on two occasions to indicate a temporal sequence, where by prior mixing along the transition zone, both PHMP-derived and DLVL-derived melt types are referred as "primary" in contrast to the secondary hybrid lavas resulting from mixing. I did not mean to imply that any of the lavas reported in my paper did not suffer any fractionation prior to eruption, and thus be "primary" in O'Hara's sense. In fact I clearly stated that they are porphyritic in texture, and explained plausible mechanisms by which lavas, once segregated from the residual mantle phases after partial melting during diapiric rise, would accumulate in magma chambers at very shallow depth beneath the thin oceanic crust, cool and begin to fractionally crystallize in order to produce the observed phenocrysts, as well as mix.

Elsewhere⁶ I also used for synonyms⁷ to "primary" the words "principal" melt types, "end-member" melt types, in referring to both PHMP-derived and DLVL-derived tholeiites.

Although we apparently agree that most of these lavas have suffered some crystallization prior to eruption, the question remaining in dispute, I believe, is quantitatively: to what extent are these melts residual, and from what mantle source or sources were they originally derived?

(2) "Olivine-gabbro and eclogite fractionation". I have shown on a $[Yb]_{EF}/[La/Sm]_{EF}$ diagram⁸ the difficulty of bridging by fractional crystallization melts with light RE-depleted pattern (typically mid-ocean ridge basalts, or Reykjanes Ridge south of 61°N) to melts with light RE-enriched pattern (typically island tholeiites, as Iceland), while at the same time keeping $[Yb]_{EF}$ relatively constant. This is shown in Fig. 1*a* and *b*. Only very large amounts of extraction (greater than 90%) of clinopyroxene with rather restricted partition coefficient values (and for that matter also of bulk chemistry) could perhaps bridge both types. This is because operative RE partition coefficients for orthopyroxene, olivine and plagioclase feldspar upon fractional crystallization do not change appreciably the $[La/Sm]_{EF}$ of residual liquids, but merely increase the overall absolute RE abundances (see also Figs 13, 15 of ref. 8). Only clinopyroxene is able significantly to fractionate and enrich residual melts in the light RE. On a $[La/Sm]_{EF}/[Yb]_{EF}$ diagram (Fig. 1*b*) the olivine-gabbro liquid-control fractionation line would have to be intermediate between individual controlling lines for plagioclase, olivine and clinopyroxene, and its exact position would depend on the relative proportions of these minerals simultaneously being extracted at the cotectic (see arrow I in Fig. 1*b*). It is clear (Fig. 1*b*) that increasing olivine-gabbro fractionation toward Iceland cannot satisfactorily produce the observed $[La/Sm]_{EF}/[Yb]_{EF}$ variation along the ridge. This, therefore, eliminates one of O'Hara's contentions, that is, an increasing olivine-gabbro fractionation toward Iceland could produce the observed increase of the $[La/Sm]_{EF}$.

So the low pressure olivine-gabbro fractionation can only increase the scatter of $[La/Sm]_{EF}$ towards Iceland, and should be inferred only as a second order effect, as is the eclogite fractionation effect which I now discuss.

Hitherto, I had avoided consideration of the effect of garnet fractionation (although aware of its effect in depleting the heavy rare earths in residual melts having fractionally crystallized garnet⁹) because of the lack of reliable garnet-melt partition data relevant to basalt genesis under eclogitic conditions. In view of O'Hara's remarks, it is necessary to illustrate the control line of liquid descent for eclogite fractionation, using Philpotts's best estimate¹⁰ set of RE partition coefficients for garnet, from the Kakanui region. This set of RE partition coefficient values for garnet gives consistent results with other estimates from other eclogites or garnet peridotites¹⁰. The set is also close to an estimate I had independently made and which is conservative on its effect in depleting the heavy RE. The effect of fractionally crystallizing garnet alone is to deplete drastically the melt in heavy rare earths such as Yb, without changing the $[La/Sm]_{EF}$ appreciably (see Fig. 1*b*). The garnet effect of lowering the $[Yb]_{EF}$ dominates also if garnet and clinopyroxene simultaneously fractionally crystallized from the melt as a 50-50% mixture as

required by O'Hara's eclogite fractionation model¹¹. Again, and contrary to O'Hara's belief, there is no way to match the observed rare earth variation along the Reykjanes Ridge with such an eclogitic fractional crystallization scheme.

Only a mixture composed of 95% clinopyroxene and 5% pyrope garnet could possibly bridge the rare earth pattern variation, but would require some 95% extract of such an unusual mixture. This is of course unacceptable, because the residual liquid of such an extract would have a major element chemistry quite distinct from an olivine tholeiite (the most common lava type over the Reykjanes Peninsula). It would also create a serious volume problem over and above having to explain the two to four times larger flux production of melt in the Iceland Region relative to more normal ridge segments, judging from crustal thickness².

(3) "Extent of fractionation". Although it is true that there is a tendency for increasing fractionation for lava ascending through thicker crust (or altitude), it is untenable that the general trace element geochemical trend observed along the ridge can be accounted for by increasing fractional crystallization alone. As previously noted on the $[La/Sm]_{EF}/[Yb]_{EF}$ diagram, the effect merely increases diversity and scatter about the general trend but cannot be responsible for it. Although at a first glance it may seem that altitude can be correlated with fractionation (such as reflected by the $[La/Sm]_{EF}$), a simple plot of relevant data would show that it is not a regular and simple function. A corollary of my plume model would be that basalt erupted south of 60°N be DLVL-derived and remain light RE-depleted, even though water depth continues generally to increase along the ridge axis. A series of five new rock analyses for stations spaced from 60°N up to the Charlie Gibbs Fracture Zone (~53°N) shows a constant level for the $[La/Sm]_{EF}$ just as south of 61°N (ref. 2, Fig. 3); and therefore further corroborates my model for two mantle sources of different densities and differentiation history.

O'Hara's preferred model of a single mantle source at the upper mantle-transition zone boundary^{1,12} offers little substance for discussion here, as it does not take into consideration the many geophysical-geochemical and geological features which contrast Iceland with the Mid-Atlantic Ridge. I do wish to emphasise that any alternative to my model should also satisfy these many model requirements, which I have listed and have taken into consideration in my model².

Contrary to O'Hara's belief, I rejected the model of a single mantle source with increasing degree of fractional crystallization toward Iceland not only on the basis of isotopic ratios (evidence which for two mantle sources is overwhelming¹³), but also from rare earth considerations as outlined above and which I had to omit in my original discussion² for the sake of brevity.

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Cavitation as a Mechanism for the Synthesis of Natural Diamonds

LET us grant, in accordance with Galimov's proposals¹, that cavitation can occur when flowing magma in a pipe encounters a constriction, and that in the ensuing collapse of a bubble very substantial transient dynamic pressures, of magnitude sufficient to be of thermodynamic importance for diamond synthesis, can be produced. In granting this, we overlook some quantitative details in his calculation, such as the apparent implication that the bubbles would contain gas at 10 or 20 kbar, and yet that their compression (by a factor of 64,000 in volume) can be calculated by ideal gas theory. He ignores the fundamental difference in rate control between martensitic conversion of crystals from one modification to another, which makes a product of the same chemical composition as the starting material, and other processes of crystal growth requiring a composition change. In the former class, to which production of diamond by the action of shock waves on graphite belongs, the limit on growth velocity is essentially the shock wave velocity. Thus, so far as that is concerned, quite a large diamond might be made within a few microseconds. Further characteristics of martensitic processes are, however, that the product takes the form of thin lenses, whereby the constraint by the matrix on shape change in the converting region is minimised and, second, that as a rule there is a multiplicity of orientations of the martensitic product in the parent crystal, so that a microcrystalline product results.

If the product is not to have the same chemical composition as the starting material, time must be allowed for diffusive segregation of chemical constituents. Galimov estimates the duration, t_{coll} , of dynamic pressure due to bubble collapse as 2×10^{-3} s. In this time diffusive segregation could occur through a thickness of order $x = (Dt_{coll})^{1/2}$, where D is the diffusion coefficient. With a fairly generous allowance of $10^{-4} \text{ cm}^2 \text{ s}^{-1}$ for D we have $x \sim 4.5 \mu\text{m}$. The thickness of diamond which could grow from one seed in the given time could not exceed this and would be proportionately less in a medium of low concentration of carbon. Of course, repeated cavitations could give repeated periods of growth, but there is no apparent reason why re-solution would not occur in the much longer intervening periods of diamond instability. Galimov offers no estimate of the carbon content of the medium, except to indicate that 30% is too much. (There are, incidentally, no grounds whatever for his idea that a supersaturated solution of carbon from which diamond could grow would need a carbon concentration of this order of magnitude.)

Thus it is conceivable that some microdiamonds are produced under dynamic pressures due to cavitation in a fast flowing magma, either embedded in graphite or otherwise: and whereas the former might survive it is highly unlikely that the latter would. But it is quite certain that the recognised diamonds for which diamond mines are worked were not produced by this process. These diamonds, of fairly equant habit, and of dimensions of millimetres to centimetres as well as smaller, frequently show inescapable evidence of growth in successive shells on all sides, with discontinuities in the growth conditions, but continuity through thicknesses of tens or hundreds of micrometres, as well as (usually terminal) periods of resolution²⁻⁶. These internal variations of impurity content and texture are readily explained by growth from solutions in times of at least the order of an hour, but quite inexplicable in terms of production by transient dynamic shock pressure.

In brief, the sole basis of Galimov's claim, in connection with his postulated mechanism, that "there are no limitations involving the total concentration of carbon in the medium" is his complete disregard of such limitations, which are inescapable.

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BIOLOGICAL SCIENCES

A Novel Substituted Guanidine with High Activity *in vitro* against Rhinoviruses

THE number and diversity of the viruses which infect the upper respiratory tract, and cause the common cold, virtually preclude the control of this disease by conventional vaccines. From this, apart from symptomatic therapy, the only hope for effective treatment of this most prevalent of diseases is in the development of broad spectrum antiviral drugs. We have developed a substituted guanidine, ICI 65,709 (ref. 1) (Fig. 1) which has good activity *in vitro* against all twenty-five rhinovirus serotypes tested in human embryonic lung cells, and also against certain other picornaviruses.

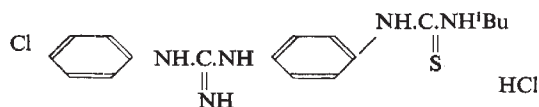


Fig. 1 The structural formula of ICI 65,709; 1-*p*-chlorophenyl-3-(*m* isobutylthioureido) phenyl guanidine hydrochloride.

The method of testing for antiviral activity has been fully described^{2,3}. In brief, it consists of infecting tissue-culture cells in 3 × 0.5-inch tubes with 100 TCD₅₀ of test virus, and then adding the test compound in a range of concentrations. After incubation at 33° C for 2 d, the cells are inspected with a low power microscope and the concentration of compound which causes 50% cytotoxicity and that which causes 50% inhibition of virus growth (cytopathic effect) are determined. From these values a tissue-culture 'therapeutic ratio' is calculated for each virus tested.

Table 1 shows that although the range of viruses tested was limited, it is clear that the activity of the compound is not confined to the rhinoviruses. Certain other picornaviruses were sensitive, and there was also some activity against vaccinia, herpes simplex, and the two arboviruses tested. The compound was inactive against equine rhinovirus, respiratory

syncytial virus and the 229E strain of coronavirus, and also against influenza or parainfluenza grown in calf kidney cells.

A curious feature of the compound was that its antiviral action was highly dependent on the type of cell in which it is tested. For example, although the growth of rhinovirus type 2 in human embryonic lung cells is strongly inhibited by ICI 65,709, the same virus grown in KB cells, HeLa or primary rhesus monkey kidney cells is completely insensitive to the compound. When rhinoviruses are grown in fragments of human embryonic trachea, 1 μg ml⁻¹ ICI 65,709 inhibits growth by approximately 90%.

Table 1 50% Toxic and 50% Antiviral Concentrations of ICI 65,709 Against Various Viruses

Cell type	50% toxic concentration (μg ml ⁻¹)	Virus	50% antiviral concentration (μg ml ⁻¹)	Therapeutic ratio <i>in vitro</i>
Human embryonic	6	Rhinovirus type 6, 5, 35	0.4	15
		33, 3, 44, 40, 1A, 9, 43	0.2	30
		32, 14, 29, 27, 11, 1B, 2	0.1	60
		15, 16, 31, 4, 26, 36, 17	0.05	120
		Equine rhinovirus	Not active	—
		Echovirus type 11	0.05	120
		14	0.1	60
		Coxsackie virus type A9	0.4	15
		A21 (Coe)	0.8	7
		B3	0.1	60
		Vaccinia	0.2	30
		Herpes viruses:		
		H. simplex type 1	1.0	6
		H. simplex type 2	1.0	6
Primary calf kidney	12	Pseudorabies	Not active	—
		Semliki forest	0.8	7
		Sindbis	0.8	7
		Influenza A ₀	Not active	—
		A ₁	Not active	—
		A ₂	Not active	—
		Parainfluenza 1	Not active	—
		Respiratory syncytial	Not active	—
		Coronavirus 229E	Not active	—

Studies on the mode of action of ICI 65,709 using rhinovirus type 2 in human embryonic lung cells have shown that the compound does not destroy the rhinovirus virion, nor does it prevent the attachment or penetration of virus particles into the host cell. When the compound is added to virus-infected cells at various times after infection, and the virus yields at the end of the first growth cycle are measured, the compound is seen to exert progressively more inhibition of virus growth the longer it is present on the infected cell. This suggests an effect on a virus synthetic process which begins soon after eclipse and continues throughout the replicative process: possibly virus RNA or protein synthesis. The compound has no inhibitory effect on the RNA or protein synthesis of the host cell at concentrations up to 100 times the 50% antiviral level.

Although ICI 65,709 is a derivative of guanidine, its antiviral action differs from that of guanidine⁴ in at least two ways. First, it does not give rise to drug-resistance as does guanidine, and second, its action is not reversed by substances such as ethanolamine, choline, methionine, or lactalbumin hydrolysate⁵⁻⁸. Biochemical studies (Koliass, S. I., and Dim-

mock, N. J., unpublished) also suggest that ICI 65,709 differs in its mode of action from guanidine.

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C-reactive Protein-like Precipitins in Plaice

C-REACTIVE protein (CRP) is an acute phase protein appearing in the serum of man during the febrile stage of infections with pneumococci and various other microorganisms^{1,2}. The protein can also be detected in serum and other serous fluids during pathological conditions in which tissue injury, inflammation or carcinoma are involved^{3,4}. Acute phase proteins have also been detected in the sera of a number of other vertebrates, including monkeys, rabbits and mice^{5,6}, but there seem to be few reports of these proteins in lower vertebrates⁶.

In the presence of Ca²⁺, CRP is precipitated from solution by pneumococcal C-substance^{1,7} and also by extracts from a number of fungi, invertebrates and plants⁸⁻¹⁰. Following the demonstration that various phosphate monoesters inhibit the CRP-C-polysaccharide precipitation reaction¹¹, phosphorylcholine has recently been found to be the most active inhibitor of all the compounds so far tested¹².

Using extracts from *Diplococcus pneumoniae*, some fungi and a nematode, *Ascaris lumbricoides*, together with a peptido-polysaccharide isolated from the dermatophyte *Epidermophyton floccosum*, we have found a CRP-like serum component in the plaice (*Pleuronectes platessa* L.), a marine teleost. We describe here specificity studies carried out with the plaice precipitin and discuss the origin and possible biological role of such proteins in poikilotherms.

Fish were caught in shallow water off the coast of Aberdeen and transferred to aerated seawater at 11–14° C in the aquarium. Single serum samples were collected¹³ between 1 h and 4 months after the capture of the fish. Human serum containing CRP was obtained from the Brompton Hospital, London, from patients with pulmonary diseases. The presence of CRP in the human samples was established by precipitation with rabbit anti-CRP (Behringwerke). Aqueous extracts of *D. pneumoniae*, *E. floccosum*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Trichophyton mentagrophytes*, *T. rubrum* and *A. lumbricoides* were prepared from culture filtrates and minced worms by methods already described^{1,8,14}.

Precipitation studies with con A have shown that this reagent reacts with polysaccharides from a variety of fungi including *E. floccosum* and a number of *Aspergillus* and *Trichophyton* species

(B. A. B. and J. Pepys, unpublished). A peptido-polysaccharide was isolated from *E. floccosum* by passage of the aqueous extract through a column of Sepharose-con A (Pharmacia) and elution of the adsorbed material with 0.1 M α -methyl-mannopyranoside in physiological saline. After dialysis and lyophilisation, the peptido-polysaccharide was used in precipitation studies.

Forty-four of forty-seven different plaice sera examined (from thirty males and females of approximately 2 to 7 yr and seventeen O-group plaice) precipitated in gel with *E. floccosum* extract. The sera also precipitated with the peptido-polysaccharide and the lines were completely confluent with those formed with culture filtrate extracts from the five fungi examined and extracts from *A. lumbricoides* and *D. pneumoniae*. The precipitin reactions observed when plaice serum and human CRP-containing serum were examined in gel diffusion experiments with some of the extracts are shown (Fig. 1a and b). As with the plaice serum, the human serum formed a continuous line of precipitation with all of the extracts. When the plaice and human sera were examined side by side in gel with *E. floccosum* peptido-polysaccharide, *E. floccosum* culture filtrate extract and pneumococcus extract, precipitation occurred in front of each of the peripheral wells and the precipitin lines merged with no sign of spurring (Fig. 1c).

Our evidence supports the view that the precipitins in the plaice sera are not antibodies. Immersion of the gels in 5% sodium citrate or 0.1 M EDTA in physiological saline caused the disappearance, within 30 min, of the lines between the plaice serum and human CRP and the pneumococcus, fungal and *Ascaris* extracts. In contrast, citrate or EDTA did not affect the precipitin reaction observed between *Vibrio anguillarum* extract and serum from a plaice previously immunised with this antigen¹⁵. Immunoelectrophoresis of whole plaice serum in Ionagar at pH 8.6 followed by development against *E. floccosum* culture filtrate extract or peptido-polysaccharide revealed a precipitin line in the α_2 -region, and antibody activity was associated with an immunoglobulin migrating in the β -region. The plaice and human CRP precipitin lines were dissolved within 2 h after immersion of the gels in 0.001 M phosphorylcholine in physiological saline. The lines were not soluble in 0.002 M solutions of choline chloride, α -D-glycerophosphate, AMP, CMP, UMP, glucose-1-phosphate, glucose-6-phosphate or in phosphate-buffered saline. Precipitation of plaice serum with *E. floccosum* peptido-polysaccharide and inhibition of this reaction with phosphorylcholine was confirmed with quantitative studies. In a typical experiment (Table 1), 200 μ l of plaice serum produced maximum precipitation (2.6 μ g of protein N) with 1,086 μ g of *E. floccosum* peptido-polysaccharide. Using these quantities of reagents, inhibition studies revealed that precipitation could be partially inhibited (76%) by 1 μ mol of phosphorylcholine and completely inhibited by 10 μ mol.

Table 1 Precipitation of *Epidermophyton floccosum* Peptidopolysaccharide by Plaice Serum

Peptido-polysaccharide used (μ g)	N in precipitate (μ g)
108.6	0.42
217.2	0.76
543.0	1.90
760.2	2.30
1,086.0	2.55
1,303.2	2.42

Quantitative precipitin analysis was carried out using a micro-precipitin technique and ninhydrin procedure for nitrogen determination²¹. Volume of serum per tube, 200 μ l. Total volume, 350 μ l.

The finding of CRP-like precipitins in the sera of apparently healthy male, female and young plaice, together with the fact that these precipitins were detected in almost all of the fish examined, suggests that this protein may be a normal component of plaice serum and not arise, as in higher vertebrates, only after infection or trauma. Some of the plaice examined had been in the aquarium for 4 months and were feeding

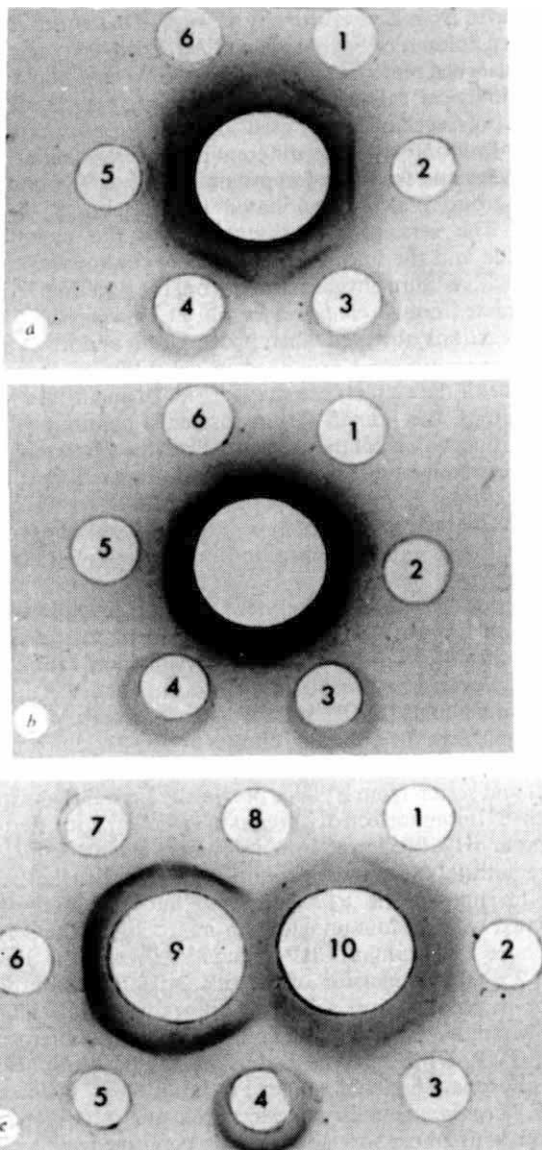


Fig. 1 Agar gel diffusion patterns formed from the reactions of plaice serum and human serum containing C-reactive protein with extracts from some fungi, *Ascaris lumbricoides* and *Diplococcus pneumoniae*. *a*, Centre well, plaice serum; peripheral wells, 1, *E. floccosum* peptido-polysaccharide 10 mg ml⁻¹; 2, *E. floccosum* extract 20 mg ml⁻¹; 3, *A. lumbricoides* extract 20 mg ml⁻¹; 4, *A. fumigatus* extract 20 mg ml⁻¹; 5, *T. mentagrophytes* extract 20 mg ml⁻¹; 6, *E. floccosum* peptido-polysaccharide 10 mg ml⁻¹. *b*, Centre well, human serum containing C-reactive protein; peripheral wells as for *a*. *c*, Peripheral wells 1, 3, 5, and 7, *E. floccosum* extract 23 mg ml⁻¹; 2, 6, *D. pneumoniae* extract 20 mg ml⁻¹; 4, 8, *E. floccosum* peptido-polysaccharide 11 mg ml⁻¹. Centre wells, 9, human serum containing C-reactive protein; 10, plaice serum.

regularly, which would suggest that they had recovered from the possible initial trauma of capture. Our preliminary studies have shown that sera from dab (*Limanda limanda* (L.)) and cod (*Gadus morrhua* L.) also contain fungal precipitins soluble in 5% sodium citrate solution. Specificity studies on these precipitins are in progress.

The ability of poikilotherms to mount a potent antibody response is now well established¹⁵ but it is also clear that antibody production is often restricted at low temperatures^{16,17}. In its natural environment the plaice is commonly found at temperatures of 10° C and below and at these lower temperatures non-antibody precipitins might provide a valuable adjunct to the animal's defences against infection. In these circumstances it would be advantageous for primitive vertebrates to have

serum proteins with broad specificity for determinants commonly found in the cell walls or surface structures of invading organisms. Phosphorylcholine may be such a widely occurring determinant as its presence in antigens from a number of bacteria, fungi and parasites has recently been suggested by precipitation results obtained with mouse anti-phosphorylcholine myeloma proteins^{18,19}. The possibility therefore exists that non-antibody precipitins form part of the fishes' humoral defences against invasion by microorganisms and parasites. Evidence of a possible anti-bacterial role for human CRP has recently been presented²⁰.

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Inhibition of Local Graft-versus-Host Reaction by Anti-Alloantibodies

THE variable part of an antibody molecule can be used as an antigen within the species that produces it. Antibodies against such variable part antigenic determinants (idiotypes) are called anti-idiotypic¹. One example of anti-idiotypic antibodies is found in alloantibody systems, where A anti-B antibodies directed against histocompatibility antigens can be shown to elicit the formation of anti-alloantibodies when injected into (A × B) F₁ hybrids^{2,3}. That the hybrid indeed synthesises anti-alloantibodies has been demonstrated both by studies on the kinetics of formation⁴ and on the allotypic specificity⁵ of the F₁ product. Such anti-alloantibodies can be shown to

react not only with humoral alloantibodies³, but also with cells carrying receptors (recognition structures) of the same idiotypic specificity². The possibility of using anti-alloantibodies for highly selective immunosuppression of transplantation reactions *in vivo* is of obvious importance. We have therefore attempted to prevent a local graft-versus-host (GvH) reaction in rats by anti-alloantibody.

Rats of the inbred strains Lewis and DA, differing at a major histocompatibility locus⁶, and their hybrids were used. Lewis anti-DA and DA anti-Lewis alloantisera were obtained 24 d after grafting DA skin on Lewis hosts and *vice versa*. These sera served to immunize two groups of 5 to 8 week old (Lewis × DA) F₁ animals. Each animal received five intradermal injections of 0.2 ml of alloantiserum in the skin of its belly on day 0, and 1.0 ml of the same alloantiserum intraperitoneally on day 14. An average of 2 ml of blood was obtained from half of the animals in each group on day 21, to be used later in passive immunisation experiments. Later on the same day 5×10^6 viable normal Lewis spleen and lymph node cells were injected into one hind foot, and the same numbers of DA lymphoid cells were injected into the other hind foot of each immunised F₁ hybrid. 7 d later, the popliteal lymph nodes were excised and weighed⁷.

Table 1 Weights (mg) of Popliteal Lymph Nodes of (Lewis × DA) F₁ Rats Actively Immunized with Lewis Anti-DA Alloantibody

Lewis node	DA node	Ratio Lewis/DA	Mean log ratio ± s.e.
12.35	82.00	0.15	-0.361 ± 0.041 *
26.84	98.32	0.27	
26.89	95.56	0.28	
24.22	82.00	0.30	
31.57	99.23	0.32	
26.27	75.54	0.35	
50.42	123.61	0.41	
35.57	86.49	0.41	
31.01	75.03	0.41	
49.25	114.93	0.43	
38.21	87.02	0.44	
53.52	117.51	0.46	
33.38	73.00	0.46	
34.22	67.48	0.51	
44.31	79.43	0.56	
64.00	105.29	0.61	
67.30	95.66	0.70	
43.92	62.09	0.71	
33.18	42.33	0.78	
48.27	58.46	0.83	

Viable lymphoid cells from strains Lewis and DA were injected into opposite feet. The corresponding popliteal lymph nodes were excised 7 d later.

* Significantly different from 0 ($P \leq 0.01$).

Table 1 shows that in F₁ animals immunised with Lewis anti-DA the node responding to the injection of Lewis cells was significantly smaller than the node corresponding to the injection of DA cells. Conversely, in animals immunised with DA anti-Lewis, the DA node was smaller than the Lewis node (Table 2). Non-stimulated lymph nodes in rats of the same age, however, averaged 7 mg and were thus considerably smaller than the "inhibited" nodes in Tables 1 and 2, indicating that this inhibition was relative but not complete.

We concluded from this experiment that in animals actively immunised with alloantibody, the GvH reaction induced by parental lymphoid cells was specifically reduced by anti-alloantibody. Other interpretations, however, might be offered. It could be argued that the injected A anti-B alloantibody itself covered certain antigenic B sites in the F₁ host, thereby impeding stimulation of A cells by these antigens.

To test this alternative hypothesis, passive immunisation with variously absorbed anti-alloantisera was arranged. Serum pools obtained from the immunised F₁ animals used in the first experiment will be referred to as anti-(Lewis anti-DA) (Table 3) and anti-(DA anti-Lewis) (Table 4), respectively.

Both sera were twice absorbed with 20×10^6 (Lewis × DA) F₁ lymphoid cells ml⁻¹ to eliminate possible remnants of the alloantibodies used to immunize the animals. Each serum was then subdivided into three parts of 4 ml each. One part was left untreated, A, the other part was absorbed three times with 20×10^6 Lewis lymphoid cells ml⁻¹ for 15 min, B, and the third part was similarly absorbed with DA lymphoid cells, C. Each sample was then injected intraperitoneally into four normal (Lewis × DA) F₁ hosts (1 ml per rat). Four hours later, 5×10^6 viable parental lymphoid cells were injected into the hind feet of each passively immunised animal, as done in the first experiment. After 7 d, the popliteal nodes were excised and weighed.

Table 2 Weights (mg) of Popliteal Lymph Nodes from (Lewis × DA) F₁ Rats Actively Immunized with DA Anti-Lewis Alloantibody

Lewis node	DA node	Ratio Lewis/DA	Mean log ratio ± s.e.
72.40	73.34	0.99	0.240 ± 0.047 *
59.00	54.34	1.09	
80.20	71.62	1.12	
44.38	37.86	1.17	
60.60	49.88	1.21	
48.31	36.39	1.33	
75.01	52.79	1.42	
69.20	48.24	1.43	
86.76	61.03	1.44	
89.21	62.00	1.44	
61.30	34.85	1.76	
59.33	33.44	1.77	
94.13	53.30	1.77	
55.04	30.09	1.83	
72.50	36.65	1.98	
81.48	33.58	2.43	
72.31	22.18	3.26	
82.51	22.41	3.68	
100.22	15.00	6.68	

Viable lymphoid cells from strains Lewis and DA were injected into opposite feet. The corresponding popliteal lymph nodes were excised 7 d later.

* Significantly different from 0 ($P \leq 0.01$).

Table 3 Weights (mg) of Popliteal Lymph Nodes of (Lewis × DA) F₁ Rats Receiving Anti-idiotypic Antibodies made in (Lewis × DA) F₁ Rats Against Lewis Anti-DA Alloantibodies

A, F₁ animals passively immunised with anti-(Lewis anti-DA) absorbed with F₁ cells only.

Lewis node	DA node	Ratio Lewis/DA	Mean log ratio ± s.e.
17.16	124.00	0.14	-0.508 ± 0.184 *
15.74	98.29	0.16	
66.25	108.75	0.61	
68.09	99.75	0.68	

B, F₁ animals passively immunised with anti-(Lewis anti-DA) absorbed with F₁ cells and with Lewis cells.

Lewis node	DA node	Ratio Lewis/DA	Mean log ratio ± s.e.
92.98	105.24	0.88	0.035 ± 0.043 †
76.15	76.32	1.00	
89.40	77.69	1.15	
119.48	87.16	1.37	

C, F₁ animals passively immunised with anti-(Lewis anti-DA) absorbed with F₁ cells and with DA cells.

Lewis node	DA node	Ratio Lewis/DA	Mean log ratio ± s.e.
52.43	101.54	0.52	-0.187 ± 0.034 *
81.57	120.45	0.68	
59.25	86.58	0.68	
56.40	76.61	0.74	

Viable lymphoid cells from strains Lewis and DA were injected into opposite feet. The corresponding popliteal lymph nodes were excised 7 d later.

* Not significantly different from Table 1; significantly different from 0 ($P < 0.05$).

† Not significantly different from 0; significantly different from Table 1 ($P < 0.05$).

Tables 3 and 4 show that the inhibition of GvH reactivity could be passively transferred with antiserum, even though this serum had been obtained from F₁ animals and had, in addition, been absorbed with F₁ cells and was thus presumably free of the last traces of the originally injected alloantibody (groups A of Tables 3 and 4).

Comparison of groups A in Tables 3 and 4 with Tables 1 and 2 shows that passive immunisation led to lymph node weight ratios not significantly different from those obtained in actively immunised rats. Absorption with cells from the strain against which the original alloantibody was directed (Table 3C and Table 4B) likewise failed to produce a significant change in weight ratio from that of actively immunised rats. In contrast, absorption with lymphoid cells from the strain which had produced the alloantibody abolished the inhibitory activity, making the mean lymph node weight ratios (Tables 3B and 4C) close to one. Such a ratio of one is found in the present strain combinations when using the same number of normal parental cells into normal F₁ hybrid recipients. This, then, must mean that the lymphoid cells used for absorption in Tables 3B and 4C carried receptors of the same idio type as the humoral alloantibody.

We interpret these findings in the following manner. Inhibition of the local GvH reaction by anti-alloantibodies cannot be explained in terms of residual alloantibody coating relevant target sites. Rather, an effect on the specific receptors for antigen on the parental lymphoid cells has to be envisaged. It is therefore likely that circulating alloantibodies induced by an allogeneic skin graft and certain receptors active in local GvH reaction share idiotypic determinants.

Our data support and extend the finding that a lethal GvH disease could be prevented by antiserum directed at recognition structures of attacking cells⁸. "Selective immunological disarmament"⁹ would thus seem to be feasible *in vivo*. This is also confirmed by two recent abstracts^{10,11} and by unpublished observations (S. Zakarian, personal communication).

Table 4 Weights (mg) of Popliteal Lymph Nodes of (Lewis × DA) F₁ Rats Receiving Anti-idiotypic Antibodies made in (Lewis × DA) F₁ Rats Against DA Anti-Lewis Alloantibodies

A, F₁ animals passively immunised with anti-(DA anti-Lewis) absorbed with F₁ cells only.

Lewis node	DA node	Ratio Lewis/DA	Mean log ratio ± s.e.
90.09	63.66	1.41	0.299 ± 0.060 *
110.13	61.43	1.79	
131.05	52.83	2.48	
127.73	51.29	2.49	

B, F₁ animals passively immunised with anti-(DA anti-Lewis) absorbed with F₁ cells and with Lewis cells.

Lewis node	DA node	Ratio Lewis/DA	Mean log ratio ± s.e.
87.21	78.86	1.11	0.115 ± 0.033 *
90.76	73.54	1.24	
87.25	64.79	1.35	
94.48	60.64	1.56	

C, F₁ animals passively immunised with anti-(DA anti-Lewis) absorbed with F₁ cells and with DA cells.

Lewis node	DA node	Ratio Lewis/DA	Mean log ratio ± s.e.
88.07	120.49	0.73	0.007 ± 0.056 †
126.51	129.67	0.98	
116.70	105.26	1.11	
129.37	96.30	1.34	

Viable lymphoid cells from strains Lewis and DA were injected into opposite feet. The corresponding popliteal lymph nodes were excised 7 d later.

* Not significantly different from Table 2; significantly different from 0 ($P < 0.05$).

† Not significantly different from 0; significantly different from Table 2 ($P < 0.05$).

Finally, from the present data we cannot decide whether B and T cells reactive against the same histocompatibility antigens use receptors of the same idio type. Purified B and T cell populations will have to be used to decide the issue. Meanwhile all that can be said is that the local popliteal lymph node assay has been considered to measure in a rather exclusive manner the reactivity of specific T cells⁷.

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Differential Staining of Nucleolus Organisers in Mammalian Chromosomes

THE *in situ* DNA-RNA hybridisation techniques to locate a certain genome fraction with unique nucleotide sequences¹⁻⁶ have assigned ribosomal cistrons to the satellites of human D and G group chromosomes^{4,5}. This is of particular interest because the satellites organise nucleoli in somatic⁷ and meiotic cells⁸⁻¹⁰. During the course of study on the Giemsa banding mechanism (unpublished) we found that the satellite bodies of human acrocentrics can be differentially stained with Giemsa after simple procedures including extraction of both nucleic acids and histones. The staining profile, herein referred to as 'N band', differed clearly from the Quinacrine, Giemsa, Reversed-Giemsa, Centromeric, or Giemsa 11 banding patterns^{11,12}. In rat kangaroo chromosomes, the N bands appeared exclusively in the nucleolus organisers^{13,14}, that is, in the secondary constriction of the X chromosome. Further application of this technique to other mammalian species strongly indicated that the N-band-positive sites coincide with nucleolus organisers.

Air-dried or flame-dried chromosome slides were prepared from various cell sources: cultured human lymphocytes, a subline of the rat kangaroo (*Potorous tridactylis*) cell line Pt-K₁, an Indian muntjac (*Muntiacus muntjak*) cell line KLS (established in our laboratory), rat (*Rattus norvegicus*) lung fibroblasts, a Chinese hamster (*Cricetulus griseus*) cell line DON, monkey (*Macaca fascicularis*) lung fibroblasts, embryonic mouse (*Mus musculus*) cells and mouse testis

cells^{15,16}. The slides were incubated in 5% trichloroacetic acid (TCA) for 30 min at 85–90° C, briefly rinsed in tap water, and reincubated for 30–45 min at 60° C in 0.1 N HCl. The slides were then thoroughly rinsed in tap water and stained for 60 min with phosphate-buffered Giemsa (diluted 1:10), pH 7.0. Digestion of chromosomes with DNase (100 $\mu\text{g ml}^{-1}$ for 60 min at 37° C at pH 6.6) and RNase (pancreatic RNase A; 100 $\mu\text{g ml}^{-1}$ for 60 min at 37° C at pH 7.0) could be substituted for the TCA step.

In well spread human metaphase plates, the N bands appeared as distinctive purplish red spots restricted to the satellite regions of all acrocentric chromosomes (Fig. 1), whereas no such deeply stained bands were observed in any other chromosome or chromosomal region including the secondary constrictions of chromosomes 1, 9 and 16. Application of the Quinacrine staining method¹⁷ before the N staining showed unequivocally that N bands were located exclusively on the satellites but not on the centromeres, short arms or stalks of satellites (Fig. 2a, b). It was obvious that even negatively or faintly fluorescent satellites could be visualised by the present technique. Although two N-band spots on each acrocentric were usual (Fig. 2b), they were frequently fused to form one spot (Fig. 1). The variation in size and number of N bands in different acrocentric members as well as in different individuals apparently reflects the genetic polymorphism which may serve as an excellent genetic marker. On the other hand, in almost all nuclei of interphase human lymphocytes the N bands appeared as tiny spots clustering within the nucleoli (Fig. 3a). Such clustering was conspicuous until late prophase when the chromosomes began metaphasic orientation (Fig. 3b).



Fig. 1 Metaphase chromosomes of a human male shown by the N-staining method.

In the rat kangaroo metaphase chromosomes, N bands were clearly visible at the secondary constriction of the X chromosomes (Fig. 4a), apparently coinciding with the nucleolus organisers^{13,14}. Indian muntjac and monkey chromosomes were also tested, as they have conspicuous secondary constrictions in certain chromosomes^{18–20}. Although their nucleolus organisers have not been located in prophase nuclei, the exclusive detection of N bands in the secondary constrictions (Fig. 4b) and interphase nucleoli was notable. In the rat and Chinese hamster chromosomes the telomeric ends and centromeres of certain bi-armed and telocentric elements were shown to be nucleolus organisers

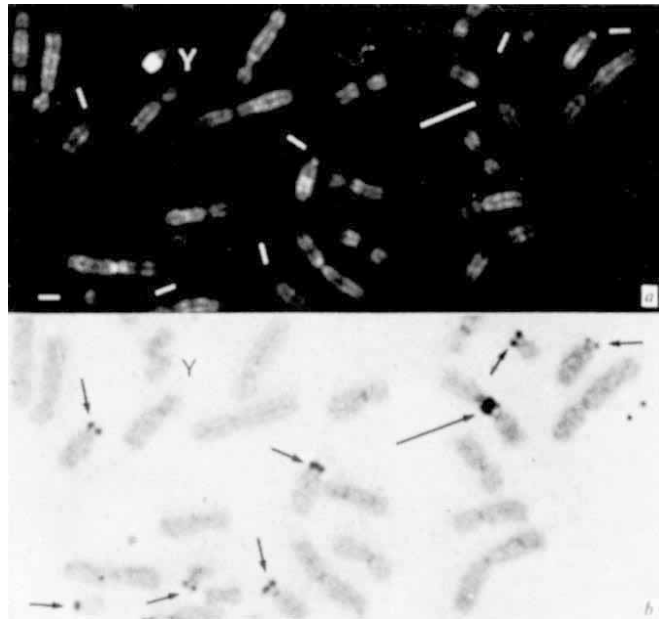


Fig. 2 A partial male karyotype of man, by Quinacrine staining (a) and N-staining (b) methods. A satellite association (upper right, b) is clearly demonstrated (long arrow).

in the prophase nuclei, as in other species^{13,21}, and to be N-band-positive. In the mouse chromosomes, however, the N band was similar in appearance to the C band, though the former appeared to be smaller and located at the centromeric end (Fig. 4c). In Sertoli cells of the mouse testis, the C-band-positive heterochromatins²² were entirely N-band-negative (Fig. 4d). As in the case of human cells, interphase cells of all the animals tested here showed deeply stained N-band figures clustering exclusively within the nucleoli. The above observations strongly suggest that in these animals all N-band-positive sites coincide with nucleolus organisers.

Since our technique was devised to extract both nucleic acids and histones in histological preparations²³, the N-band substances were thought to be acidic proteins. This was supported by several tests. First, chromosomes treated with TCA and HCl were all negative for Feulgen, methyl green-pyronin, alkaline fast green (pH 8.0)²⁴, ammoniacal silver²⁵ and PAS stainings. Second, chromosomes digested with proteolytic enzymes such as Pronase (Kaken, Tokyo; 100 $\mu\text{g ml}^{-1}$ for 10 min at 37° C) or trypsin (Sigma; three times crystallised; 10 $\mu\text{g ml}^{-1}$ for 10 min at 37° C) did not show the N band. Third, treatment with 0.1 N NaOH for 1 min

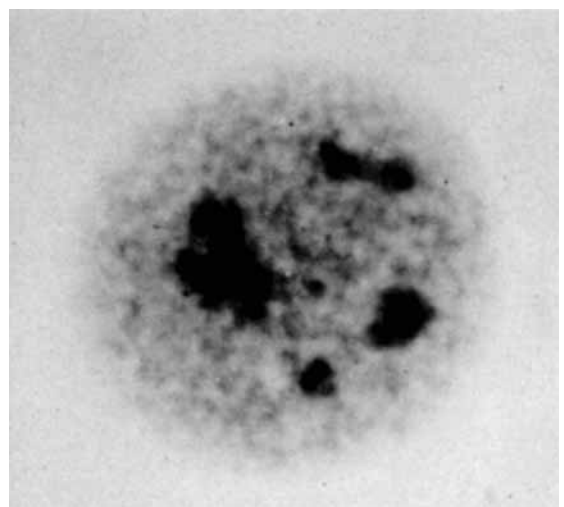


Fig. 3 Cultured human interphase lymphocyte stained by the N-banding method.

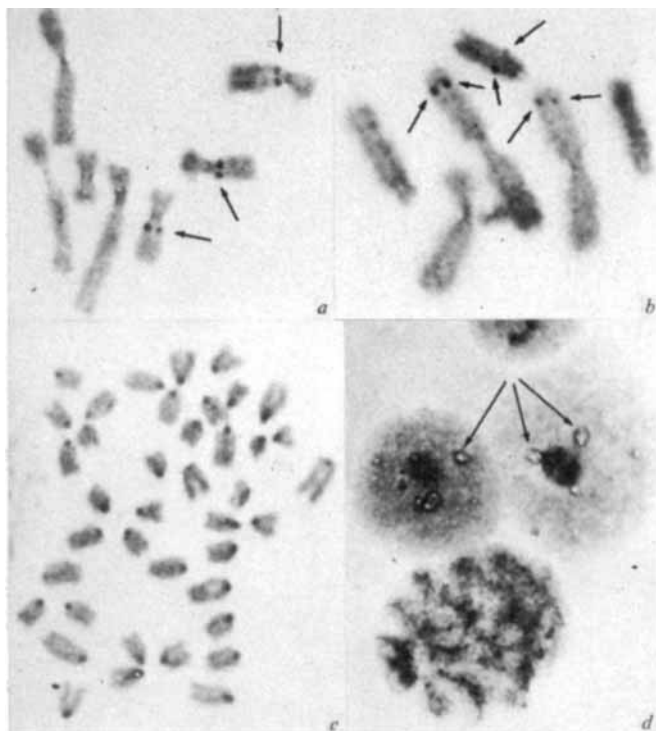


Fig. 4 N bands from: *a*, rat kangaroo (partial metaphase of a triploid subline of Pt-K₁ with three X chromosomes); *b*, Indian muntjac; *c*, embryonic cell of the mouse; *d*, mouse testis, showing negatively stained C bands in Sertoli cells (arrowed). The N-band cluster is obvious in the nucleoli.

after the TCA and HCl steps abolished N-band staining, whereas treatment with 0.2 N H₂SO₄ after the TCA and HCl steps did not change N-band staining.

Although the mechanism involved in the N-staining procedure remains uncertain, it is interesting that acidic proteins may be involved in the expression of ribosomal cistrons in mammalian nuclei. N bands may provide useful genetic markers, and we are investigating further their cytogenetic and biochemical properties.

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Why Centric Regions of Quinacrine-treated Mouse Chromosomes show Diminished Fluorescence

Rowley and Bodmer¹ reported that the centric regions of quinacrine-treated mouse chromosome preparations show less fluorescence intensity than the remainder of the chromosome. They argued that because these centric regions contain a type of DNA that is richer in AT base pairs than unique sequence DNA, they would be expected to show diminished fluorescence according to the model proposed by Caspersson *et al.*², according to which localised quinacrine fluorescence was attributed to the preferential binding of quinacrine mustard to the N-7 position of guanylyl residues in DNA. Thus, one would expect a relative paucity of guanylyl residues in the DNA of the centric regions to be associated with diminished fluorescence. It has since been shown^{3,4} that enhanced quinacrine fluorescence is due to AT base pairs, and studies of a series of GC-containing DNA polymers indicated that GC base pairs (or G alone) actually quenched fluorescence⁴. These observations did not explain why the centric regions of mouse chromosomes with their relatively greater abundance of AT base pairs show diminished quinacrine fluorescence.

Table 1 Effect of DNA Polymers on Quinacrine Fluorescence

DNA polymer added	mol % G+C	Fluorescence	Fold over control
None	—	35	0
(dA) _n (dT) _n	0	85	+1.4
(dG) _n (dC) _n	100	10	-0.7
(dA) _n (dT) _n +(dG) _n (dC) _n (1:1 mixture)	50	55	+0.6
<i>E. coli</i> DNA	52	5	-0.9
<i>C. perfringens</i> DNA	32	30	-0.1

DNA polymers (10⁻⁴ M, phosphate basis) were mixed with quinacrine (2×10⁻⁶ M) in 0.1 M (Na) phosphate buffer, pH 6.8. Excitation was at 425 nm and fluorescence was measured at 490 nm.

To answer this question we examined further fluorometric properties of quinacrine in the presence of DNA polymers (Table 1). (dA)_n(dT)_n enhances quinacrine fluorescence while (dG)_n(dC)_n quenches fluorescence. A 1:1 mixture prepared by mixing equal volumes of these two samples still enhances fluorescence but with diminished efficiency, presumably due to dilution. In any case, the net effect is still one of enhancement by an equimolar mixture of polynucleotides which gives a final GC concentration of 50 mol%. In contrast, DNA from

Escherichia coli which contains 52 mol% GC quenches quinacrine fluorescence, as does DNA from *Clostridium perfringens* with 32 mol% GC.

These observations suggest that the degree of fluorescence enhancement is not strictly a function of the AT/GC ratio but that the degree of interspersion of GC base pairs among AT base pairs plays an important role. Thus, in a comparison of two polynucleotides with the same base composition, the one which has a higher degree of GC base pair interspersion would be expected to show a greater degree of quenching. A corollary would be: for two polynucleotides with different base compositions, the polynucleotide with less GC could actually quench more if the GC base pairs were more evenly interspersed (compare *C. perfringens* DNA and $(dA)_n \cdot (dT)_n + (dG)_n \cdot (dC)_n$). An extreme example of GC interspersion would be one in which GC (and therefore AT) base pairs were distributed evenly throughout the length of the polynucleotide. Such DNA would be highly repetitive, a well known property of the DNA found at the centric regions of mouse chromosomes.

Caspersson and Zech⁵ originally proposed that preferential binding to accessible sites is the determinant of quinacrine fluorescence. In a recent work⁵ the possibility of quinacrine fluorescence enhancement by AT-groups and quenching by GC groups is acknowledged. On the other hand, our fluorometric studies of dyes that bind to DNA lead us to the conclusion that for those dyes which have useful fluorescence staining properties for chromosomes, the fundamental determinants of the fluorescence pattern seen are the bases and their relation to each other; both the primary and secondary structures can play a role. Whether the fluorometric properties of the dye are actually expressed may depend on secondary factors such as accessibility, controlled by unidentified hypothetical proteins. The results described here exemplify a further application of this principle.

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Cytosine Arabinoside and Rosette Formation in Mouse Retina

Glücksman and Tansley¹ reported that the developing retina was very vulnerable to X rays and this has been confirmed by many other investigators²⁻⁴. Some drugs which inhibit DNA synthesis may also inhibit cellular proliferation and thus cause maldevelopment of the retina. This would result because, in the neural retina of the suckling animals, active cellular proliferation is taking place producing neurones at the inner and outer nuclear layers^{5,6}.

Cytosine arabinoside, a common antitumour drug, inhibits DNA polymerase^{7,8}. Because of this, it is used in the treatment of some viral infections, such as herpesvirus infection⁹⁻¹¹ and cytomegalic inclusion disease¹². We have investigated the possible effects of this drug on the developing mouse retina.

One hundred and twenty-one suckling mice, ICR-JCL

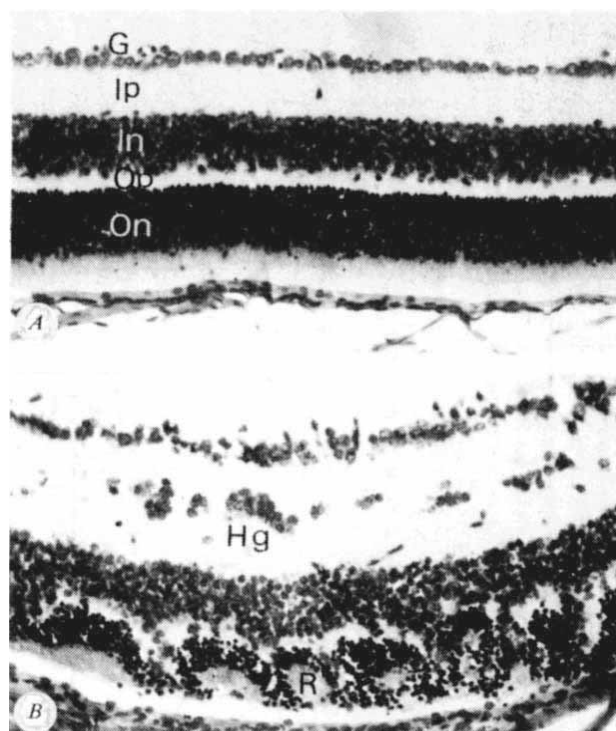


Fig. 1 A, Normal retina of 20-d-old mouse. G, Ganglion cell layer; Ip, inner plexiform layer; In, inner nuclear layer; Op, outer plexiform layer; On, outer nuclear layer. B, Retina of 20-d-old mouse which had three successive doses of (50 mg kg⁻¹) cytosine arabinoside on postnatal days 2, 3 and 4. R, Rosette; Hg, heterotopic ganglion cell in the inner plexiform layer.

strain, were divided into two groups. Forty-seven mice were injected subcutaneously with cytosine arabinoside (30 mg kg⁻¹) 2, 3 and 4 d after birth (group 1). Seventy-four animals were similarly treated with 50 mg kg⁻¹ (group 2). Treated animals of both groups were then killed 5, 7, 10, 15, 20 and 30 d after birth. The eyes were removed, fixed in either Bouin's or Carnoy's solution, embedded in 'Paraplast' and cut into 6 μ m sections. The sections were stained either with haematoxylin-eosin or 1% cresyl violet. Retinas of untreated mice of the same age were used as controls.

Twenty-four hours after the final injection of cytosine arabinoside the undifferentiated nuclear layer was seen to be selectively destroyed and to contain a large quantity of nuclear debris. Large cavities resulting from the necrosis of undifferentiated cells were also prevalent in this layer. In the retina of 5-d-old controls many large, round, pale nuclei, which later formed the inner nuclear layer, were seen at the inner zone of the undifferentiated nuclear layer. On the outer surface of this layer, however, mitosis was still common. When the experimental animals were examined on postnatal day 7, the nuclear debris and cavity in the nuclear layer had disappeared although many rosettes were found, either scattered or in rows, at the outer nuclear layer. A few cells in mitosis were found on the inner surface of this structure. By this time the retina of a 7 d old control was already similar to that of an adult. Even 20 and 30 d after birth, rosettes were found in 81 to 67% of the retinas in group 1 and in all of group 2 (Table 1). They were especially numerous on the portion of the retina close to the ora serrata. The ganglion cells of the treated mouse were arranged irregularly and the inner plexiform layer was approximately twice as wide as that of the controls. Within the inner plexiform layer, heterotopic cells, which are morphologically similar to ganglion cells, were found in clusters or in rows (Fig. 1).

Table 1 Incidence of Rosettes in the Retinae of Treated Mice

Group	No. of animals treated	Age when killed (d)												No. killed	30																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																													
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The animals belonging to group 1 were given three successive doses of cytosine arabinoside (30 mg kg⁻¹). The animals in group 2 were given three successive doses of 50 mg kg⁻¹. +, One to four rosettes; ++, five to ten rosettes; +++, more than ten rosettes.

The total effect on the retina caused by the administration of cytosine arabinoside to the suckling mouse is summarized in Table 1. Total doses used in this experiment are not very different from those used clinically for the treatment of leukaemia or viral infection. The results therefore indicate the possibility that such treatment may cause lasting damage to the developing retina.

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Modulation of Lymphocyte Receptor Redistribution by Concanavalin A, Anti-mitotic Agents and Alterations of pH

VARIOUS lymphocyte surface receptors can be redistributed after cross linkage by external ligands, leading to the formation of patches and caps¹. At 21° C and 37° C concanavalin A (con A) in concentrations greater than 5 µg ml⁻¹ inhibits patch and subsequent cap formation induced by anti-immunoglobulin and other reagents^{2,3}. Con A can also induce cap formation by its own receptors under certain conditions, notably after binding at 4° C^{3,4}. Thus con A has two antagonistic activities in modulating lymphocyte surface receptor distribution and their expression depends on the conditions of incubation. We now report results suggesting that colchicine-binding proteins⁵ (possibly those of the microtubules) are important in the modulation of cell surface mobility. In addition, we have found that alterations of pH affect the ability of lymphocytes to form patches and caps.

Splenic lymphocytes from NCS mice (Rockefeller University) were used. The preparation of fluorescein-labelled con A (fl-con A) has been described elsewhere². Unless otherwise noted, Hanks' balanced salt solution (HBSS) containing 5% (v/v) foetal bovine serum was the medium.

After lymphocytes had been incubated with 100 µg ml⁻¹ fl-anti-immunoglobulin at 37° C for 10 min, about 45% of the total cells were stained and 85% of them showed caps; however, if 100 µg ml⁻¹ con A was added before the fl-anti-immunoglobulin, 45% of cells were stained but only 1%

showed caps. If the cells were pretreated for 1 h at 37° C with 10⁻⁴ M colchicine, an anti-mitotic agent⁶ which dissociates microtubules⁵, 30% of those exposed to con A and fl-anti-immunoglobulin showed typical patch formation followed by cap formation. Because colchicine did not induce cap formation, it must have partially suppressed the inhibitory effect of con A on patch and cap formation with fl-anti-immunoglobulin (Fig. 1). Similarly, incubation with 100 µg ml⁻¹ fl-con A at 37° C for 15 min showed that 95% of cells were stained, with only 1–2% showing caps, but 20% or more cap-forming cells were observed when cells were pretreated with 10⁻⁴ M colchicine.

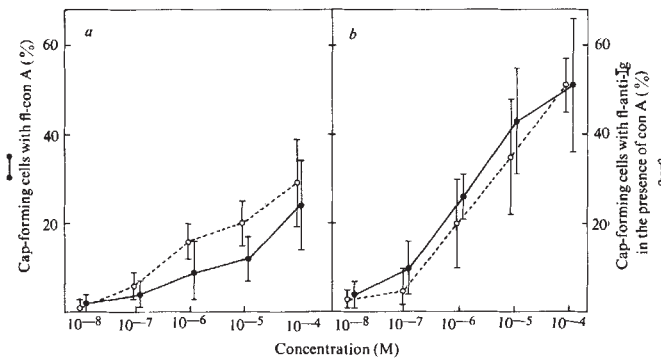


Fig. 1 Effect of colchicine (a) and vinblastine (b) on inhibition of patch and cap formation by con A. Cells were incubated separately with various concentrations of colchicine and vinblastine at 37° C for 30 min and incubated for another 10 min with 100 µg ml⁻¹ fl-con A (●) or with 100 µg ml⁻¹ fl-anti-immunoglobulin in the presence of 100 µg ml⁻¹ con A (○). Cap formation was determined as described previously⁸.

Time of incubation with colchicine was not critical, for the drug affected cap formation with fl-con A even if both were added at the same time. Furthermore, when cells were incubated with fl-con A at 37° C for 15 min, washed, and then incubated at 37° C for 1 h in the presence of 10⁻⁴ M colchicine, 25% of cells formed caps. In contrast, if colchicine was omitted, only about 2% of cells showed caps.

Similar but more marked effects were observed using the *Vinca* alkaloid, vinblastine⁷ (Fig. 1). Other anti-mitotic agents including colcemid, vincristine⁷, and podophyllotoxin⁸ also suppressed the inhibitory effect of con A (Table 1). The relatively weak effect of podophyllotoxin may be related to its impurity as well as its limited solubility in the medium. Griseofulvin⁸ did

not influence the inhibition of patch and cap formation by con A; however, some commercial samples of griseofulvin are reported to lack anti-mitotic activity⁸. Although colchicine, vinblastine and strychnine affect erythrocyte membrane structure⁹, strychnine had no obvious effect in our system. Lumicolchicine¹⁰, a structural isomer of colchicine that does not bind to colchicine-binding proteins and is devoid of anti-mitotic activity, had no effect at concentrations comparable with those used with colchicine (Table 1).

To test the reversibility of the colchicine and vinblastine effects, cells were incubated with each drug at 37° C, washed, incubated for various times at 37° C and tested for cap formation (Fig. 2a). The effect of colchicine persisted for at least 2 h without significant decrease, and even after 6 h, 13% of cells had caps. The colchicine effect therefore seemed to be only partially reversible. In contrast, the vinblastine effect seemed to be almost fully reversible (Fig. 2a). The decrease in the number of cells forming caps with fl-con A after removal of vinblastine was not due to the intrinsic loss of ability to form caps, for there was no decrease in the number of cells forming caps with fl-anti-immunoglobulin during similar periods (Fig. 2b).

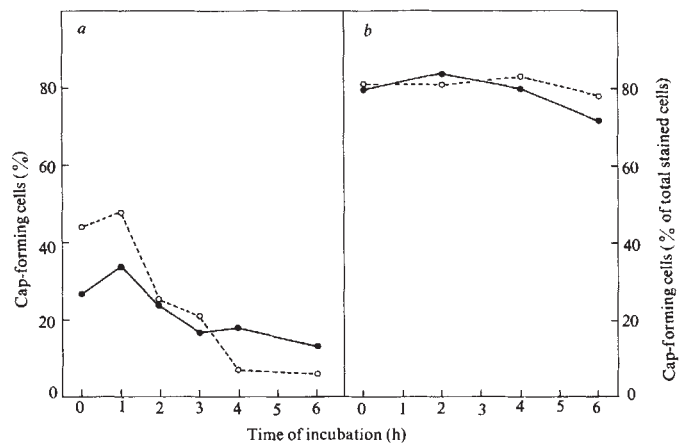


Fig. 2 Reversibility of colchicine and vinblastine effects. a, Cells were incubated separately with 10⁻⁴ M colchicine (●) and 10⁻⁴ M vinblastine (○) at 37° C for 30 min, washed twice and incubated at 37° C for various times in the medium containing no drug. The cells were subsequently incubated with a, 100 µg ml⁻¹ fl-con A; b, 100 µg ml⁻¹ fl-anti-immunoglobulin at 37° C for 10 min.

Low temperatures¹¹ and high hydrostatic pressures¹² dissociate microtubules. Although our efforts to demonstrate an effect of increased hydrostatic pressures yielded variable results, we found that low temperatures affect the number of cap-forming cells⁸. If splenic lymphocytes were incubated with 100 µg ml⁻¹ fl-con A at 4° C, washed in the cold and then incubated at 37° C, approximately twenty times as many cap-forming cells were observed than after incubating cells at 37° C with 100 µg ml⁻¹ con A. This cannot be explained by the fact that three times as many con A molecules are bound at 37° C than at 4° C, for the differences were observed even when identical numbers of molecules were bound at the two temperatures.

Our results suggest that the integrity of microtubular structures may be required for inhibition of patch formation by con A and that the mobility of cell receptors is modulated by structures associated with or related to microtubules. The concentrations of colchicine and vinblastine effective in our experiments are considerably greater, however, than those required to inhibit mitosis or disrupt microtubular structures visible by electron microscopy⁸. Moreover, microtubules with a diameter of 250 Å have never been observed either in or just under the cell membranes of lymphocytes. It is possible, therefore, that

Table 1 Effect of Anti-mitotic Drugs on the Con A Effect

Drug	Concentration (M)	Caps (%)* with fl-con A†	Caps (%)* with fl-anti-immunoglobulin and con A‡
—	0	1 ± 1	1 ± 1
Colchicine	10 ⁻⁴	24 ± 10	30 ± 8
Colcemid	10 ⁻⁴	25	20
Vinblastine	10 ⁻⁴	51 ± 12	51 ± 6
Vincristine	10 ⁻⁴	19	15
Podophyllotoxin	10 ⁻³	8	10
Lumicolchicine	5 × 10 ⁻⁴	3	1
Griseofulvin	10 ⁻⁴	1	2
Strychnine	10 ⁻³	4	1

*The percentage of the population showing cap formation was counted using samples of 100 cells or more. In cases where more than ten determinations were carried out, the values are given with standard deviations.

† 100 µg ml⁻¹ fl-con A.

‡ 100 µg ml⁻¹ fl-anti-immunoglobulin and 100 µg ml⁻¹ con A.

the system affected by colchicine resembles the 250 Å microtubules but differs from them in organisation. Cationic alkaloids, such as vinblastine or strychnine, can precipitate with actin-like proteins isolated from various cells¹⁴. Although strychnine had no effect in our system, actin-like proteins may form structures that modulate the mobility of lymphocyte receptors. As colchicine, vinblastine, vincristine and podophyllotoxin bind to microtubules^{8,15} and because conditions that disrupt microtubules also affect the inhibition of patch formation by con A, a colchicine-binding protein resembling tubulin seems a more likely candidate than other proteins or phospholipids.

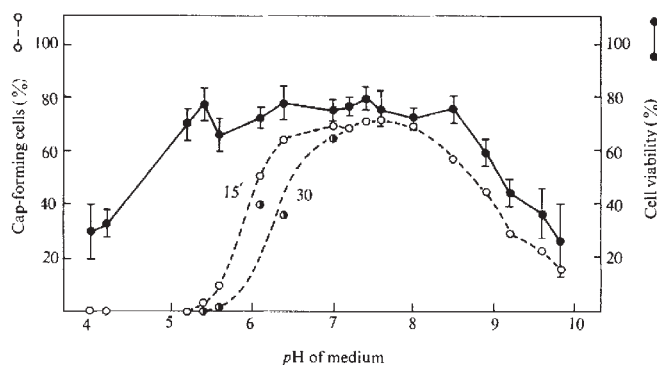


Fig. 3 Effect of pH on patch and cap formation. The following buffered isotonic media were used: 10 mM acetate-buffered saline (pH 4.2, 5.4), 10 mM citrate-phosphate-buffered saline (pH 4.0, 5.2, 5.6, 6.1, 6.4, 7.0), 10 mM Tris-HCl-buffered saline (pH 7.2, 7.6, 8.0, 8.4, 8.8), 10 mM glycine-NaOH-buffered saline (pH 8.5, 8.9, 9.2, 9.6, 9.8), PBS (pH 7.4), HBSS (pH 7.2). Cells were pre-incubated separately in those media at 37° C for 15 min and then incubated with fl-anti-immunoglobulin at 37° C for 10 min in the same media. Cell viability (●) was determined by the trypan blue dye exclusion; bars represent standard deviations of repeated measurements. Cap (○) formation was determined as described previously. The effect on cap formation with fl-anti-immunoglobulin is also shown after prolonged incubation (30 min) of cells in acidic media (○).

The observations reported here may be related to reports that colchicine affects both the agglutination induced by con A and phagocytic events in polymorphonuclear leukocytes^{16,17}, suggesting that the topographical distribution of con A receptors and transport sites for small molecules are controlled by microtubules. The differences we observed in reversibility of the effects of colchicine and vinblastine are similar to those obtained relative to the movement of mouse peripheral macrophages¹⁸.

So far only con A^{1,19}, other mitogenic lectins²⁰ and high concentrations of sugars¹⁹, such as 0.5 M D-mannose, have been shown unequivocally to affect patch formation reversibly. Loor *et al.*¹⁹ reported that LaCl₃ inhibits patch formation induced by anti-immunoglobulin. We could not repeat their experiments because we were unable to prepare 1–10 mM LaCl₃ solutions in media containing phosphate ions at neutral pH without precipitation or lowering of the pH of the medium. Moreover, 10 mM LaCl₃ in 10 mM Tris-HCl-buffered saline (pH 7.0) resulted in cell clumping with a drastic reduction in cell viability and precipitation of fl-anti-immunoglobulin.

We have found that cap formation induced by fl-anti-immunoglobulin was inhibited at both acidic and alkaline pH. A sharp decrease in the number of cap-forming cells was observed at pH 6.1 to 5.2 and neither patch nor cap formation was observed at pH 5.2. When cells were incubated for prolonged periods, the critical pH was more alkaline (Fig. 3). Because slight decreases in cell viability were observed within the effective range (Fig. 3) and the inhibition was reversible (Table 2), the decreases in cap-forming cells in acidic media cannot be accounted for by cell death. Patch formation was frequently observed in alkaline media even in cells not forming caps,

and the absence of caps paralleled the viability curve. It is likely, therefore, that the inhibition of cap formation in alkaline media is due to cell damage and to depression of cellular metabolism before cell death.

Table 2 Effects of Colchicine Treatment and Exposure to Low pH on Cap Formation

First incubation*	Second incubation†	Third incubation‡	I Caps (%) with fl-anti-immunoglobulin	II Caps (%) with fl-anti-immunoglobulin and con A	III Caps (%) with fl-con A
pH 7.2	pH 7.2	pH 7.2	77	2	3
pH 7.2	pH 5.2	pH 7.2	78	2	2
pH 7.2+ colchicine	pH 7.2	pH 7.2	81	39	31
pH 7.2+ colchicine	pH 5.2	pH 7.2	81	41	46

* Cells were incubated at 37° C for 30 min with or without 10⁻⁴ M colchicine and washed.

† Cells were incubated at 37° C for 15 min in citrate-phosphate-buffered saline (pH 5.2).

‡ Cells were incubated at 37° C for 10 min with 100 µg ml⁻¹ fl-anti-immunoglobulin in the absence (column I) or presence (column II) of 100 µg ml⁻¹ con A, or with 100 µg ml⁻¹ fl-con A (column III).

Unlike the inhibition by con A, the effect of acidic pH was not reversed even partially by colchicine. For example, when lymphocytes were treated with 10⁻⁴ M colchicine and subsequently incubated with fl-anti-immunoglobulin in medium at pH 5.2, cap-forming cells were not observed. The lack of effect of colchicine at pH 5.2 cannot be due to the release of molecules from colchicine-binding proteins¹⁵, because the effect was obvious if cells treated with colchicine and then washed were briefly incubated at pH 5.2, and returned to neutral pH (Table 2). The specific mechanism and sites of action mediating the inhibitory effect at acidic pH seemed to be different from those of con A. Patch formation is independent of metabolism^{1,2} and therefore the effects of acidic pH are not attributable to any effects of pH on metabolism. Acidic pH may affect phospholipid head groups of the membrane, the phase separation characteristics of the membrane lipids, or submembranous structures.

Our present and previous data^{20,21} suggest that both the tetravalence of con A and the integrity of a colchicine-binding structure are required for inhibition of patch formation. It is not yet clear whether con A can inhibit micropatch formation demonstrable at the electron microscopic level²² but not at the level of fluorescence microscopy. In any event, our results suggest that although cell receptors may be mobile in the membrane^{23,24} their relative mobility may be modulated by structures the exact nature of which remains to be defined. This hypothesis is compatible with observations²⁵ suggesting that some of the receptors on the cell surface penetrate the membrane possibly to interact with other cellular components.

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Crossbands of *Caulobacter crescentus* Stalks serve as Indicators of Cell Age

BACTERIA in the genus *Caulobacter* have a life cycle with two distinct cell types¹: a non-motile mother cell with a stalk extending from one cell pole, and a polarly flagellated swarmer cell which has no stalk. During the life cycle the mother cell elongates and divides to produce a swarmer at its non-stalked pole. The swarmer forms a holdfast at the base of its flagellum and, before division, produces a stalk from the same site. The synthesis of the stalk occurs at the cell-stalk junction².

Structures variously called Querbalken, crossbands, and annuli have been reported in *Caulobacter* stalks since they were first observed in the electron microscope³⁻⁶. This study was undertaken to determine how the formation of these structures is related to the life cycle of the bacterium.

Caulobacter crescentus strain CB2-L was used in all experiments. It was maintained on medium MMB⁷.

In one series of experiments synchronous cultures were initiated with swimmers. *C. crescentus* was inoculated into a sterile, 500 ml, pear-shaped separatory funnel containing MMB and 400 g of 4 mm glass beads. The culture was incubated at room temperature for 24–48 h before the synchrony experiments were begun. Then approximately 4 l of sterile MMB was washed over the beads to remove all unattached or loosely attached cells. The flow was stopped for 15 min to allow swarmer cells to be released by mother cells attached to the beads. Then 150 ml was eluted from the column into a sterile 500 ml Erlenmeyer flask that was transferred to a shaker water bath at 30° C. Ten millilitre samples were collected and fixed with Lugol's iodine at zero time and at 30 min intervals thereafter for examination with the electron microscope (AEI EM6B at 60 kV). Enlarged prints of electron micrographs were used to measure stalk length, cell length, and number of crossbands. Total counts were determined on samples every 15 min using a Petroff-Hauser counting chamber. The curve shown in Fig. 1 was obtained.

The initial population consisted of 5.9×10^6 cells per ml. Oil immersion phase and electron microscopic counts indicated that the swimmers, C_0 , comprised about 90% of the total population (91.5 and 92.5%, respectively). At 30 min some cells

had begun to synthesise stalks but the average stalk length was only 0.10 μ m. At 1 h stalk synthesis had commenced in almost all cells, and the average stalk length was 0.5 μ m. Only one stalked cell in fifty contained a crossband. By 1.5 h the range of stalk lengths was 0.55–1.40 μ m with an average of 1 μ m. About 47% of the stalked cells (twenty-two out of forty-seven) had one crossband (only one in fifty had more). Although many of the cells at 1.5 h had indentations indicating they were in the process of division, most cells separated in the succeeding half hour.

At 2 h, the average stalk length decreased to 0.9 μ m because newly formed swimmers of the next generation, C_1 , had begun to produce stalks. If it is assumed that all C_0 cells had stalks longer than 0.55 μ m (the minimum length observed in these cells at 1.5 h), then 69% (forty out of fifty-eight) had one stalk septation and the average stalk length was 1 μ m, or about the same as at 1.5 h. Therefore the rate of stalk synthesis approached zero at the time of cell division and cell separation.

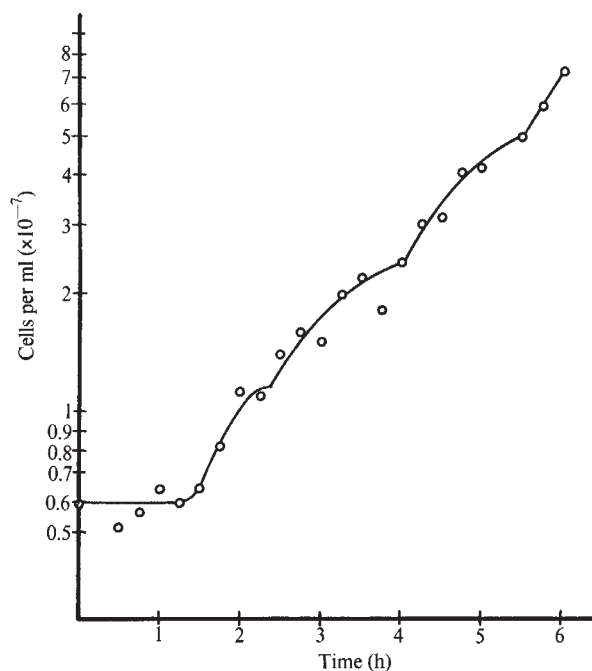


Fig. 1 Growth of a synchronous culture of *Caulobacter crescentus* CB2-L initiated with swarmer cells. Cells began dividing at about 1.5 h and had completed division by about 2 h. The second doubling was completed by 4 h and the third by 5.5 h.

At 2.5 h stalks of the C_0 population had resumed elongation and the average length was 1.25 μ m. Assuming that the minimum stalk length of these cells increased at the average rate of elongation it would be 0.8 μ m at 2.5 h: 88% of the cells with stalks that length or longer, which are presumably the C_0 population, had a single crossband. Thus, most, if not all, cells of this strain produce a crossband during or shortly after cell division. This conclusion is consistent with the independent studies of Jones and Schmidt⁸.

At 4 h, when the second doubling was completed, nineteen stalked cells had two crossbands and twenty-two had one. This is close to the 1:1 ratio expected if all C_0 cells had developed a second crossband and all C_1 cells had developed their first crossband following division. None of the fifty-two stalked cells examined had more than two crossbands at this time. At 5.5 h, when cell numbers had again doubled, cells with three crossbands were found. By this time, however, the synchrony had deteriorated considerably.

Stalk elongation continued after the first generation so that at 4 h the C_0 cells (those with two crossbands) had an average

stalk length of 1.65 μm and at 5.5 h the average stalk length of the C_0 cells, which now had 3 septa, was 1.75 μm . Apparently, during logarithmic growth, the average rate of stalk synthesis remains high for the first two generations and then decreases. We also noted that stalks with two and three crossbands were much longer (3.15 and 4.91 μm , respectively) during stationary phase than during logarithmic growth, presumably due to the effect of phosphorus limitation known to result in abnormally long stalks in *Caulobacter*².

The following picture of stalk and crossband formation best fits the above data: (1) swarmer cells may begin stalk synthesis any time after division (at one extreme we have an occasional cell undergoing separation having stalks at both exposed ends and, at the other extreme, rare cells dividing without stalks), (2) the rate of stalk synthesis decreases at the time of cell division, (3) crossband formation occurs during division, and (4) stalk elongation resumes after division.

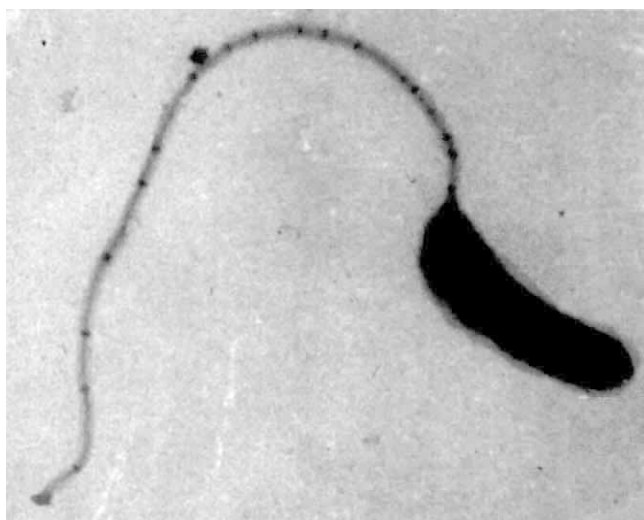


Fig. 2 A *C. crescentus* cell containing eighteen crossbands in its stalk. Most, or perhaps all, cells of this strain form a crossband in their stalks at the time of division so that the minimum age of the cell is eighteen generations.

Using another experimental approach a population of swimmers was grown in conditions of unlimited growth to determine whether crossbands were formed each generation even after numerous divisions had occurred. To accomplish this, swimmers from a logarithmic population were allowed 20–30 min to attach to a short section of sterile glass tubing. The tubing was then aseptically transferred to a siphon connected to a 20 l reservoir of sterile MMB (all at 30° C). The medium was passed through the chamber at a rate of about 40 ml h⁻¹ to remove newly formed swimmers. The section of tubing was removed after 52 h. The cells were fixed and gently dislodged from the wall of the tubing with a transfer loop and observed with the electron microscope. The estimated generation time was 2.7 h. Thus, the predicted maximum age of a cell was nineteen generations. If crossbands were formed each generation, then some cells would be expected to have nineteen crossbands. The maximum number of crossbands found was eighteen, close to the theoretical expectation (Fig. 2).

The results of this study indicate that the stalk of *C. crescentus* CB2-L continues to elongate beyond the first generation and crossbands are formed in most, if not all, cells during the time of division. It seems likely that all caulobacters which produce crossbands (all species in the genera *Caulobacter* and *Asticacaulis*) would produce them in the same manner, so that counting stalk crossbands may become a generally useful procedure for determining cell ages of bacteria in these genera. If so, the effect of cell age upon metabolism and other aspects of cell physiology can be investigated in this group of aquatic bacteria. In addition, it should be possible to follow the ageing of *Caulobacter* communities in their natural habitats.

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Histamine H₁ and H₂-Receptors at a Ganglionic Synapse

MANY humoral agents are capable of modulating synaptic transmission in sympathetic ganglia and it has been proposed that a variety of receptors are present at the ganglionic synapse (see Haefely¹). There are thus many possible modulatory mechanisms at this synapse, some of which have a dual nature, the same substance being able both to facilitate and to depress ganglionic transmission by an action on two different populations of receptors. We wish to present evidence that histamine, like the catecholamines² and 5-hydroxytryptamine (serotonin)³, has a dual action at the ganglionic synapse.

Trendelenburg^{4,5} demonstrated that histamine can facilitate transmission through the feline superior cervical ganglion *in vivo* and found that this facilitation could be prevented by the classic anti-histaminic drug, mepyramine⁶. We have observed that histamine can either depress or facilitate transmission in the rabbit superior cervical ganglion depending on circumstances. A dual action of histamine on blood pressure has been reported⁶ and it has been proposed that the pressor effects are due to activation of one type of histamine receptor, the H₁-receptor, while the depressor effects are due to activation of a second type of receptor, the H₂-receptor (J. W. Black, D. A. A. Owen, and E. M. Parsons, unpublished). Whereas H₁-receptors are blocked specifically by mepyramine, H₂-receptors are unaffected by this drug but may be blocked by burimamide (N-methyl-N-(4-(5-imidazolyl)butyl)thiourea)⁷. We wished to establish whether H₁- and H₂-receptors were present at the ganglionic synapse and whether they were responsible for the observed dual actions of histamine.

The action of histamine was investigated on the rabbit isolated superior cervical ganglion in a bath allowing continuous superfusion of the ganglion with Krebs solution at 37° C⁸. Drugs were added to the superfusate either so as to give a constant concentration or by injection of a fixed amount into the superfusion stream. Injections into the superfusion stream are dispersed in a volume of about 10 ml. Stimuli, either submaximal or maximal for eliciting the initial (Sa) component of the postganglionic compound action potential, were delivered to the preganglionic nerve trunk about 30 mm proximal to the ganglion. Postganglionic action potentials were recorded from the internal carotid nerve at least 3 mm distal to the ganglion. Sa amplitude was measured from the pre-existing baseline; in none of the experiments reported here was subdivision of the

Sa component observed. Facilitation or depression is presented in the text as a percentage of estimated control values, allowing for any progressive change in control values with time.

The postganglionic compound action potential evoked by a single stimulus (response elicited at 30 s or, more usually, 1 min intervals) was reduced in amplitude in twelve out of thirteen preparations by histamine acid phosphate given as a constant superfusion (0.33–33 μM) or by injection of 0.1–0.33 μmol into the superfusion stream; in only one preparation was the response facilitated by histamine. The mean reduction of responses to maximal stimuli by 3.3 μM histamine (1 $\mu\text{g ml}^{-1}$) was $14\% \pm 3\%$, $n=8$. The effect of similar concentrations of histamine on action potentials evoked by continuous repetitive stimulation at 2 Hz was less consistent. Repetitive stimulation at this frequency itself induces a substantial facilitation of responses to both submaximal and maximal stimuli as a result of intrinsic modulatory processes⁸; thus, responses to maximal stimuli in these experiments were facilitated by $30\% \pm 3\%$, $n \pm 5$. In contrast to its effect on single responses, histamine depressed the responses to repetitive (2 Hz) stimulation in only one of eleven preparations; in six preparations histamine had no unequivocal effect, while in four preparations a facilitation was observed. In one experiment, 33 μM histamine caused a 29% reduction of responses to single submaximal stimuli (Fig. 1a) and in the same preparation caused a transient 19% facilitation of the responses elicited by stimulation at a frequency of 2 Hz (Fig. 1b).

It seems possible that the facilitatory effect of histamine, sometimes seen when repetitive stimulation was employed, may reflect an action of the drug often masked by its depressant action. Our results suggest that the depressant actions are usually predominant when responses are evoked by single stimuli; with repetitive stimulation, perhaps as a consequence of the activation of intrinsic facilitatory processes, the depressant actions of histamine are less apparent. Mepyramine, the classical antagonist of histamine H_1 -receptors, did not antagonise the histamine-induced depression of responses to single stimuli. On the contrary, in two experiments the depression produced by 3.3 μM histamine was increased in the presence of 2.5 μM mepyramine maleate (1 $\mu\text{g ml}^{-1}$). In Fig. 2 the histamine-induced depression was increased from 16% to 38% in the presence of mepyramine. In the only experiment in which histamine facilitated responses to single stimuli, the 3–12% facilitation induced by 3.3 μM histamine was replaced by a 22% depression in the presence of mepyramine. On the assumption that the increased depression is due to antagonism by mepyramine of a concomitant facilitatory action of histamine mediated by H_1 -receptors, the characteristics of the depressant action of histamine are best studied in the presence of mepyramine. Other experiments suggested that 2.5 μM mepyramine was adequate for suppression of the facilitatory effects induced by 3.3 μM histamine. In preparations exposed continuously to 2.5 μM mepyramine, the histamine-induced depression of the evoked response was dependent on concentration and the threshold concentration was around 0.03 μM . The mean reduction of responses to single stimuli by 3.3 μM histamine in the presence of mepyramine was $25 \pm 6\%$, $n=4$.

If the histamine-induced depression is mediated by H_2 -receptors, this action should be antagonised by the H_2 -receptor antagonist, burimamide. We chose concentrations of burimamide which have been shown to cause significant blockade of H_2 -receptors in the vascular system of the rabbit and on guinea pig isolated atria⁷. In Fig. 2, it can be seen that addition of 4.7 μM burimamide (1 $\mu\text{g ml}^{-1}$) increased the rate at which recovery from the histamine-induced depression occurred after removal of histamine from the superfusion medium. Further, a subsequent superfusion with 3.3 μM histamine in the presence of both mepyramine and burimamide (4.7 μM) caused a much smaller depression (17%) of the evoked response; increasing the burimamide concentration to 47 μM almost abolished the depressant action of histamine in this experiment. In three out of five experiments, a combination of 2.5 μM mepyramine and

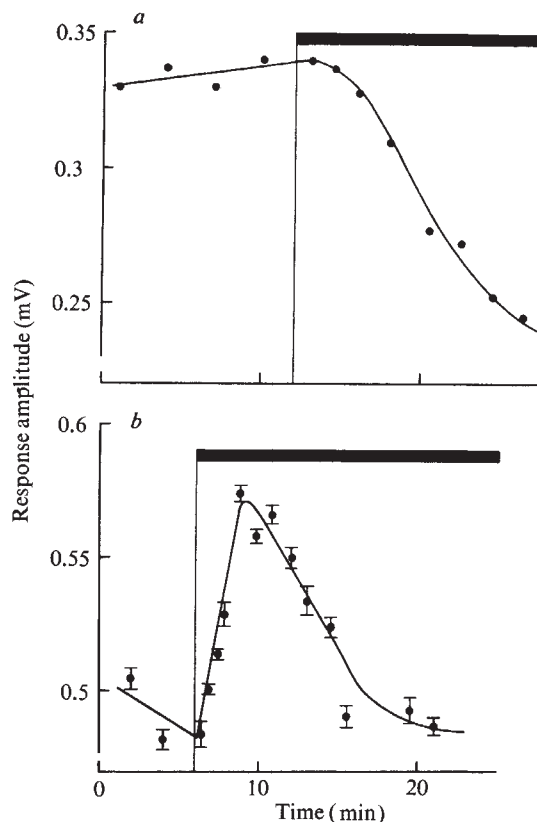


Fig. 1 Effect of histamine on amplitude of postganglionic compound action potential evoked by a single submaximal stimulus (a) or by repetitive submaximal stimulation at 2 Hz (b). Histamine (33 μM) present in perfusion medium for periods indicated by the black bars. In (a) each point represents a single observation or the mean of two or three observations. In (b) each point is the mean $\pm$ s.e.m.

47 μM burimamide completely abolished the effects of 3.3 μM histamine on responses to single stimuli; in the other two preparations a residual histamine-induced depression of about 3% remained. The presence of both blockers also completely abolished the actions of 0.1 μmol (30 μg) histamine injections into the superfusion stream.

In the absence of mepyramine, 47 μM burimamide consistently antagonised the depressant action of histamine and revealed the facilitatory action. In the experiment shown in Fig. 3, 3.3 μM histamine induced a 16% depression of the evoked response on initial superfusion and a 17% depression on a second superfusion. Burimamide (47 μM) accelerated the recovery from this second histamine-induced depression, and a third exposure to histamine in the presence of burimamide resulted in a 10% facilitation of the evoked response. The subsequent addition of 2.5 μM mepyramine to the superfusion medium completely abolished this facilitation, only a small depressant action being apparent on a fourth superfusion with histamine. In each of four experiments superfusion with 3.3 μM histamine in the presence of burimamide produced a facilitation ($10\% \pm 1\%$).

If the lack of effect of histamine on many preparations stimulated at 2 Hz is due to approximately equal facilitatory H_1 - and depressant H_2 -actions, then blockade of one type of receptor should reveal the action mediated by the other. This was the case: one preparation, which initially failed to respond to injections of 0.1 μmol histamine, responded with 7–8% facilitation of the evoked action potentials in the presence of 47 μM burimamide; in another preparation, 2.5 μM mepyramine revealed a small (4–5%) histamine-induced depression to injections of 0.1 μmol .

Neither mepyramine nor burimamide in the concentrations studied here had any detectable effect by themselves on the

amplitude of the postganglionic compound action potential. In some experiments (see, for example, Fig. 3), long-term fluctuations in action potential amplitude occurred, but these appeared to be unrelated to the addition of drugs.

On the assumption that mepyramine and burimamide are relatively specific antagonists of H_1 - and H_2 -histamine receptors⁷ respectively, the above results are consistent with the presence at the ganglionic synapse of both these receptor types, H_1 -receptors mediating the facilitatory actions of histamine and H_2 -receptors mediating the depressant actions. It is interesting that a recent report⁸ has suggested that there are two sorts of 5-hydroxytryptamine receptors at the ganglionic synapse, one mediating a depressant, the other an excitatory action. The localisation of the histamine receptors either presynaptically or postsynaptically is not possible on the available evidence, although it is known that histamine causes little or no measurable depolarisation of ganglion cells (ref. 1; and D. I. Wallis and B. Woodward, unpublished). The presence at the ganglionic synapse not only of H_1 - and H_2 -histamine receptors, but also of α and β adrenoreceptors⁹, muscarinic acetylcholine receptors, tryptamine receptors^{3,9}, γ -aminobutyric acid receptors^{10,11}, and receptors to certain polypeptides¹ is extremely puzzling. Are all these receptors merely vestigial? Do they perhaps represent an inevitable but physiologically insignificant sensitivity of the mechanisms operating at the synapse to biologically active substances or are they, on the other hand, involved in a complex

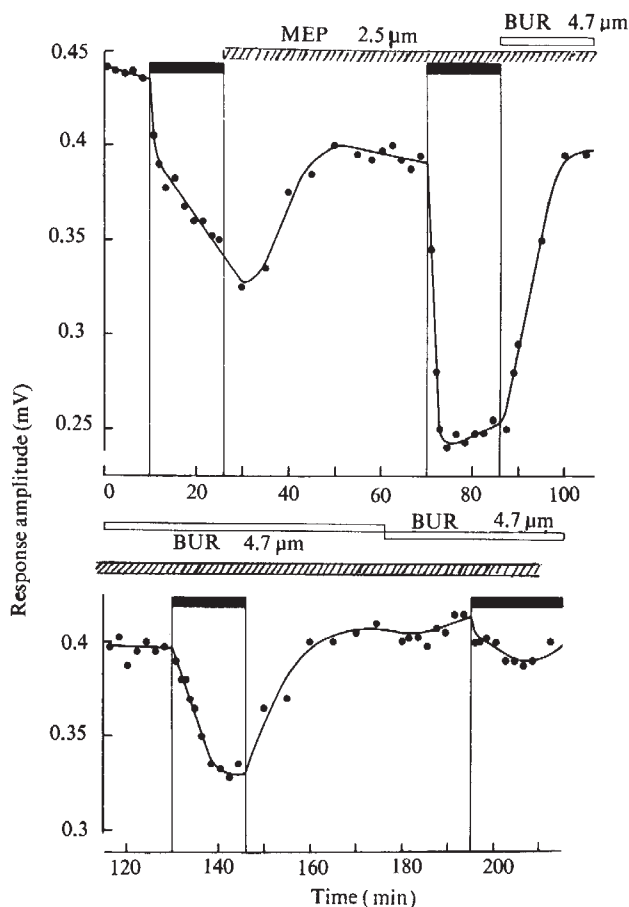


Fig. 2 The effects of mepyramine and of a combination of mepyramine and burimamide on the depressant action of histamine. Responses to single maximal stimuli. Histamine ($3.3 \mu\text{M}$) present in perfusion medium for periods indicated by the black bars. Each point represents a single observation or the mean of two observations. Mepyramine (MEP) ($2.5 \mu\text{M}$) present for the duration of the hatched bar and during the third and fourth exposures to histamine. Burimamide present during the periods indicated by the white bars, initially as a $4.7 \mu\text{M}$ solution (BUR $4.7 \mu\text{M}$) and subsequently as a $47 \mu\text{M}$ solution (BUR $47 \mu\text{M}$).

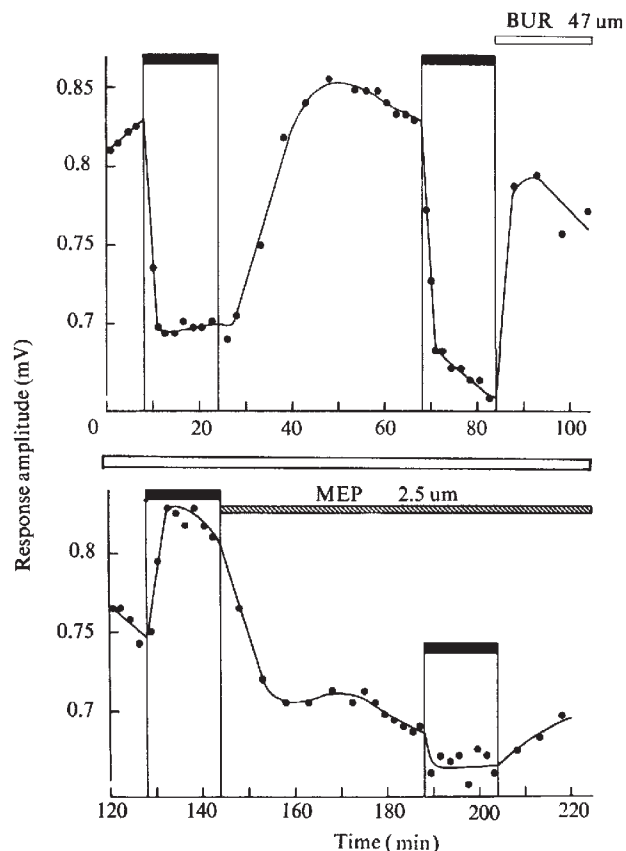


Fig. 3 The effects of burimamide and of a combination of burimamide and mepyramine on the action of histamine. Responses to single maximal stimuli. $3.3 \mu\text{M}$ histamine present in perfusion medium for periods indicated by the black bars. Each point represents a single observation or the mean of two observations. $47 \mu\text{M}$ burimamide (BUR) present for the duration of the white bar and during the third and fourth exposures to histamine. $2.5 \mu\text{M}$ mepyramine (MEP) present during the period indicated by the hatched bar.

system of regulation by which agents carried in the blood stream or released locally can exert an important influence over the transmission process?

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Role of Insulin as a 'Permissive' Hormone in Mammary Gland Development

THE growth and differentiation of the mammary gland is controlled by the synergistic action of insulin, prolactin and ovarian or corticosteroid hormones¹⁻⁴. The ovarian steroid induced alveolar development of the virgin mammary gland *in vivo* is mediated by activation of nucleic acid and protein synthesis and mitosis⁵⁻⁹. On the other hand, numerous studies using organ cultures, mostly of fragments of mid-pregnant mammary gland, emphasised the role of insulin as the mitogenic hormone in mammary gland development¹⁰⁻¹². This variable nature of responsiveness of the mammary tissue to hormones *in vivo* and in organ culture has been discussed in recent reports^{13,14}. The proliferative mammary gland of pregnancy, mostly used in these organ culture studies¹⁰⁻¹², should contain a large proportion of cells in the division cycle at the time of excision; we considered that hormonal regulation of the fate of these dividing cells during incubation *in vitro* may influence the interpretation of the results obtained from the organ culture studies. In order to test this possibility, mid-pregnant mammary cells of Balb/c mice were labelled for 1 h with ³H-thymidine (³H-dT) *in vivo*, and the transit patterns of the labelled cells reaching mitosis were determined at different times in organ culture.

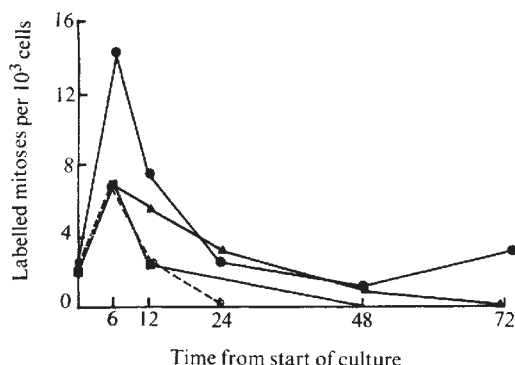


Fig. 1 Number of ³H-dT labelled mitoses per 1,000 mammary epithelial cells at different times in organ culture. The mammary gland of a 13-d-pregnant Balb/c mouse was used. Days of pregnancy were determined by the vaginal plug method. 100 μ Ci ³H-dT (49.2 Ci mmol⁻¹) (New England Nuclear Corporation, Boston, Mass.) was injected intraperitoneally 1 h before killing by cervical dislocation. Pulse labelling time of ³H-dT in mouse mammary gland *in vivo* is 30 min¹⁵. For organ culture, fragments (1–2 mm) of mammary gland were incubated at 37°C in 95% O₂ and 5% CO₂ atmosphere in plastic Petri dishes containing Waymouth (MB 752/1) medium with penicillin⁵. The organ culture medium was supplemented with 5 μ g ml⁻¹ each of insulin (▲), or insulin + prolactin (●), or prolactin (■) or was unsupplemented (○). The cultures were terminated at different times and the explants were fixed in acetic acid and ethanol (1:3 v/v) for liquid emulsion autoradiography (Kodak NTB 2)⁴. The autoradiograms of histological sections were scored under a 100 $\times$ oil immersion objective. The data represent typical values of 3–4 sets of cultures and at each time point 5,000 cells from 4–5 explants were scored. Metaphase to telophase cells were counted as mitosis.

As expected, mammary epithelial cells were initially labelled at a high frequency. The average number of silver grains per nucleus was reduced at different times of incubation, suggesting that the slight initial increase in the frequency of labelling is due to the mitotic division of some of the labelled cells rather than to continued incorporation of ³H-dT during incubation. Figure 1 shows that in I medium and in P containing insulin or prolactin alone the frequency of labelled mitoses increased modestly at 6 h, then decreased to zero, whereas in IP medium the frequency of labelled mitoses increased more than seven-fold at

6 h, decreased until 48 h, and rose again at 72 h. In NH medium, the frequency of labelled mitoses after an initial rise decreased to zero at 24 h; both in P and in NH medium extensive alveolar degeneration also occurred at this time. These results show that the mammary cells which were in the progenitor compartment *in vivo* can continue through the cell cycle reaching mitosis in organ culture. The variations in the frequency of labelled cells reaching mitosis in I, IP, or P medium at different times indicate, however, that the hormones greatly influence the cell transit time in organ culture.

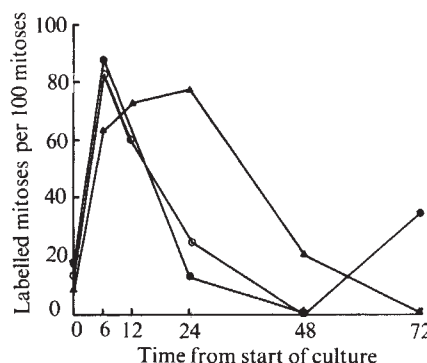


Fig. 2 Percentage of ³H-dT labelled mitoses among the mitotic cells. 100–200 mitoses were scored at each time point. ▲, Insulin; ○, prolactin; ●, insulin + prolactin. Other details as in Fig. 1.

In I medium the proportion of mitoses which are labelled increased slowly during the initial 24 h in organ culture, whereas in P and IP medium the proportion of labelled mitoses reached nearly 100% at 6 h, then dropped to zero at 48 h; in IP medium it rose again at 72 h (Fig. 2). The slow rate of increase of labelled mitoses between 6 h and 24 h in I medium strongly suggests that cells which were in the S phase *in vivo* spend a long time completing DNA synthesis *in vitro*, presumably because of a delayed recovery from the initial lag period in organ culture. The near 100% proportion of labelled mitoses at 6 h (7 h after injection of ³H-dT) in IP medium shows that mammary cells in IP medium complete DNA synthesis at a nearly normal rate (the duration of S in mid-pregnant mammary cells being 8–9 h (ref. 16)) reaching mitosis after a short initial latent period in organ culture. The unusually long S phase (20–21 h) (refs 15 and 16) of virgin mammary cells should similarly cause DNA synthesis to continue for an extended period during the recovery from the initial lag and this, rather than the earlier suggestion that virgin mammary cells acquire a delayed sensitivity to insulin in organ culture¹⁷, may be the explanation of the late rise of DNA synthesis in these cells in I medium.

Figure 3 shows that in I medium total mitosis slightly increased at 24 h but that it was below the initial value. In IP medium, a modest increase of mitosis at 6 h was followed by a two-fold rise at 12 h, a decrease to nearly zero at 48 h and another increase at 72 h. In P medium the general pattern was similar to that in I medium, but the mitotic activity was slightly higher than in the I medium until 24 h, and at 48 h it dropped to zero. In NH medium the cells failed to recover from the initial lag period and the mitotic activity declined to zero at 24 h. Since in I medium 65–75% of mitoses were labelled during the interval between 6 and 24 h (Fig. 2), the only slightly increased mitotic activity during this period indicates a negligible stimulation of new cells into mitosis by insulin in organ culture. In IP medium, since only 12% of mitoses were labelled at 24 h (Fig. 2), 88% of the cells in mitosis observed at this time must have been initiated into the division cycle during organ culture. Results in Fig. 3 further show that the addition of prolactin after 24 h incubation in insulin alone stimulated new cells into mitosis.

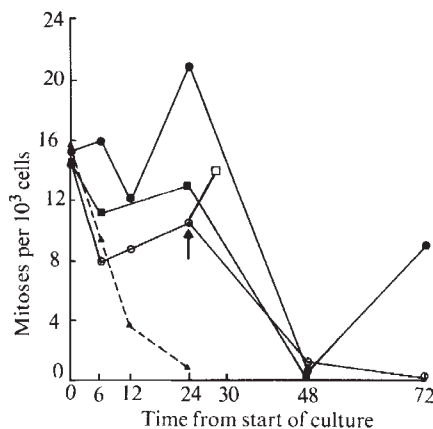


Fig. 3 Total mitotic (labelled and unlabelled) activity in mammary epithelium of mouse mammary gland in organ culture. ○, Insulin; ●, insulin + prolactin; ■, prolactin; ▲, no hormone; □, insulin 24 h, then insulin + prolactin up to 30 h. Arrow indicates the time prolactin was added. Other details are as in Fig. 1.

These observations thus show that under the conditions of the previous studies¹⁸⁻²⁰ mid-pregnant mammary cells which were in the division cycle *in vivo* should show increasing levels of ³H-dT incorporation (after 3-6 h pulse labelling *in vitro*) up to 24 h during their recovery from the lag period in I medium, which may lead to the interpretation that insulin alone stimulated DNA synthesis and mitosis in the mammary epithelial cells in organ culture^{11,12}. Our results suggest, however, that the cells which divide in the mammary tissue during the early hours of insulin-supplemented organ culture are mostly those which had already begun the division cycle in the mid-pregnant mammary gland *in vivo*. Insulin alone does not initiate new cells into proliferation, but acts as a 'permissive hormone'²¹ supporting completion of the division cycles of those cells which were initiated *in vivo*. The permissive action of insulin is, however, obligatory for the mitogenic action of prolactin in organ culture of mouse mammary tissue. Since exogenous ovarian steroids are detectable in the mammary tissue 72 h after a single injection²² the mitogenic action of insulin plus prolactin may be synergistic with the endogenous ovarian hormones carried in the mid-pregnant mammary tissue in organ culture. Contrary to earlier suggestions^{11,12} the requirement of hormones for mouse mammary cell proliferation may not be different *in vivo* and in organ culture.

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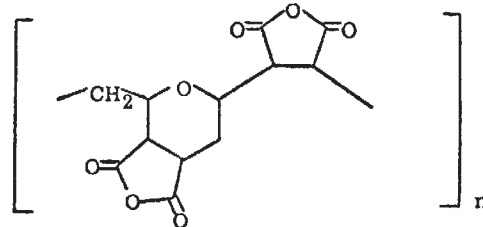
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Divinyl Ether-Maleic Anhydride (Pyran) Copolymer used to demonstrate the Effect of Molecular Weight on Biological Activity

DIVINYL ether and maleic anhydride form a 1:2 copolymer, which is reported to have the structure shown here¹. It is commonly known as pyran copolymer, although we prefer to call it DIVEMA, an acronym for divinyl ethermaleic anhydride. It has been designated as NSC 46015 by the National Cancer Institute.



The copolymer has various biological activities and has elicited considerable interest, as demonstrated by more than forty articles since 1967. DIVEMA has activity against several solid tumours, for example, adenocarcinoma², Lewis lung carcinoma³, C3H mouse mammary tumour³, and has been approved for initial clinical evaluation. It induces interferon⁴; it is active against several viruses, including Friend leukaemia^{5,6}, Rauscher leukaemia⁶, Moloney sarcoma⁷, vesicular stomatitis⁸, Mengo⁹, MM¹⁰ and foot-and-mouth disease¹¹; it has antibacterial¹² and antifungal activity^{12,13}; it stimulates immune response¹⁴⁻¹⁷; it inhibits adjuvant disease¹⁸, and it is an interesting anticoagulant¹⁹.

Several observations led us to a more thorough investigation of the structure of DIVEMA. Degraded material was found to have considerably lower acute toxicity in mice. Although most of a polymer prepared with ¹⁴C-labelled maleic anhydride was excreted by mice fairly rapidly, a portion was excreted very slowly²⁰. Munson *et al.*²¹ had shown a biphasic response of the reticuloendothelial system (RES) towards DIVEMA; initially there was a decrease in the rate of phagocytosis, followed by a considerable increase over the control after several days. Combining these observations, we hypothesised that the copolymer might consist of two materials, one toxic, very slowly metabolised, and responsible for the RES depression, and the other less

toxic, rapidly metabolised, and responsible for the RES stimulation. We assumed that the two materials would differ only in molecular weight, and that the high molecular weight portion was the undesirable material. This has now been confirmed.

To characterise the polymers, they were converted into their methyl esters by first refluxing them in methanol and then treating them with ethereal diazomethane. These esters were fractionated by gel permeation chromatography (GPC) on a Styragel column using tetrahydrofuran (THF) as solvent (similar results have been reported by Butler and Wu²⁰). A comparison of the molecular weights of the anhydride and the methyl ester demonstrated that no appreciable degradation had occurred during esterification. The intrinsic viscosities and weight-average molecular weights of fractions from a preparative GPC were determined in THF. They were then analysed on an analytical GPC column and compared with known polystyrene standards on the same column using the same solvent. A plot of the peak-elution volumes against $\bar{M}_w[\eta]$ gave a single curve (Fig. 1), $\bar{M}_w[\eta]$ being the 'universal parameter'²³. We were then able to determine the molecular weights and the polydispersities of other samples by using this calibration curve. This procedure demonstrated that the usual slurry polymerisation to high conversion²⁴ leads to polymer with broad molecular weight distribution.

Our goal was the synthesis of polymers in the desired molecular weight range with a narrow distribution, since we felt that the high molecular weight portion which would be present if the distribution were broad would contribute more to toxicity than to activity. In an attempt to obtain sufficient sample for evaluation, several large-scale fractionations of anhydride were carried out on sand columns, using a procedure developed originally for the fractionation of polyolefins²⁵. Because it was difficult to obtain enough material for evaluation by fractionation, we turned to direct polymerisation. By low-temperature photochemically initiated solution polymerisation in THF, a solvent for the copolymer at low but not high temperatures, narrow distribution copolymer could be prepared; molecular weight could be controlled to some extent by varying the monomer con-

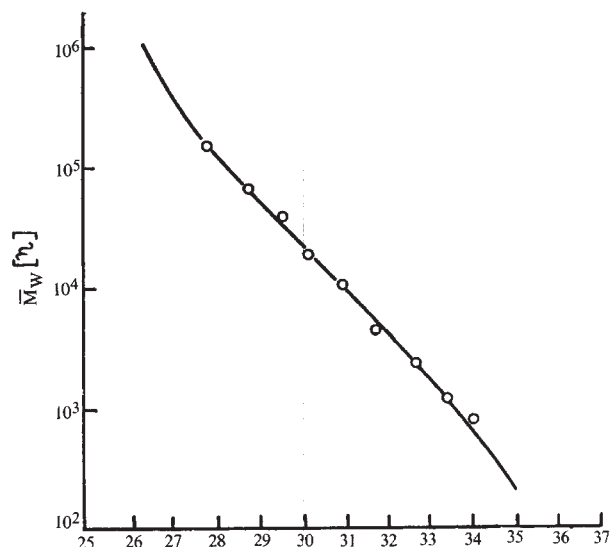


Fig. 1 Universal calibration curve for GPC using THF at 30°C. The line represents polystyrene standards, the circles pyran copolymer methyl ester fractions.

centrations. Acetone was a better solvent for the copolymer and almost any desired molecular weight could be obtained using a free-radical initiator in acetone with THF as a chain-transfer agent.

Table 1 lists different polymers in order of increasing molecular weight ($\bar{M}_p$ is the peak molecular weight from GPC of the methyl esters). NSC 46015 is a sample approved for clinical evaluation and XA 124-177 is a similar slurry polymer of higher molecular weight; both have broader molecular weight distributions than either the fractionated polymers or those made in solution. Even though the differences in acute toxicity are not large, it is apparent that

Table 1 Biological Activity of Various Samples of Pyran Copolymers

Sample	$\bar{M}_p^*$	$\bar{M}_w/\bar{M}_n^*$	Acute toxicity †	SGPT ‡	Phagocytic index §	Endotoxin sensitisation ¶ (% mortality)	Drug metabolism (% inhibition)	Antitumour Ehrlich†† (% inhibition)	Lewis lung‡‡ (T/C)
Control	—	—	—	30	0.044	0	—	—	—
A	2,500	1.7	—	34	0.105	0	—	66	>130
B	3,000	1.7	—	48	0.073	0	15	—	>171
C	3,900	2.3	—	30	—	0	—	—	>133
D	5,200	1.9	—	54	—	20	—	—	125
E	6,900	1.9	127	46	0.055	0	0**	43	—
F	14,700	1.6	131	52	0.075	0	0	61	—
G	19,600	2.2	100	93	0.014	100	30**	45	—
H	25,000	2.6	—	132	—	100	—	—	—
I	44,800	2.3	87	96	0.010	100	57**	—	—
NSC 46015	22,500	3.7	96	>200	0.021	100	67	67	>117
XA 124-177	32,200	7.2	72	>200	0.015	100	65	53	—

All tests except Lewis lung were done on white male Swiss NYLAR mice, polymer dose 25 mg kg⁻¹, given intravenously.

* From GPC of methyl ester.

† LD₅₀ intravenously.

‡ Serum glutamic pyruvate transaminase, Sigma Frankel Units.

§ From first order rate of carbon clearance, 24 h after injection.

¶ Endotoxin from *Salmonella typhosa*, 3 mg kg⁻¹ intravenous challenge.

|| Aminopyrine.

** Antipyrine.

†† Decrease in weight of tumour compared with control.

‡‡ Data obtained from Drug Research and Development, Chemotherapy, NCI; mean survival time, % of control.

toxicity as measured by the other criteria increased with increasing molecular weight. Thus, SGPT levels and inhibition of drug metabolism, both measures of liver function, increase with increasing polymer size, as does sensitisation to endotoxin. Phagocytosis is stimulated by low molecular weight polymer and depressed by high.

Most important, it was demonstrated that low molecular weight copolymers with narrow molecular weight distributions are not only low in toxicity but retain the antitumour activity shown by higher molecular weight samples against both Ehrlich adenocarcinoma and Lewis lung carcinoma in mice; the latter has proved extremely difficult to inhibit with standard chemotherapeutic agents^{26,27}.

Data available on the relationship between molecular weight and antiviral activity are limited to encephalomyocarditis in mice. The only conclusion which can be drawn at present is that with this virus a higher molecular weight is required for activity than for antitumour activity. Obviously other viruses must be examined before generalisations can be made.

In the light of the numerous literature references²⁸ to problems associated with the toxicity of pyran copolymer, the synthesis of materials with lower toxicity could be of major importance.

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Hyperthermia in Rabbits from Injection of Malaria Infected Erythrocytes in the Pre-optic Area

MALARIA, when introduced naturally by the bite of an anopheline mosquito, goes through an exoerythrocytic stage in the parenchymal cells of the liver. Here the malarial parasite matures to a mature merozoite that infects erythrocytes. When the parasite has multiplied, the red blood cell bursts and many new infective merozoites are released. These infect new erythrocytes. During lysis of erythrocytes the host develops a fever¹. It is not known whether the malarial parasite produces a pyrogen, like bacteria, or whether the malarial fever results from destruction of red blood cells.

The pre-optic area of the brain of mammals is thought to be the site of both temperature reception and integration in the central nervous system²⁻⁴. This area is far more sensitive to injected pyrogens than other sites in the brain⁵ suggesting that pyrogens interfere directly with nervous regulation of body temperature. Substantial fevers can be produced in mammals by injecting 1/100 of the effective intravenous dose of pyrogen directly into the pre-optic area⁵. Pyrogens released from leukocytes during infection are thought to be responsible for biogenic fevers, whereas pyrogens released from bacteria are scavenged from the blood and do not reach central nervous sites of temperature regulation⁶. When injected directly into the brain both classes of pyrogens induce fever, but leukocytic pyrogen acts more quickly (8 min) than bacterial pyrogens (45 to 60 min)^{1,5}. We took advantage of this differential sensitivity to test the pyrogenicity and nature of malarial parasites.

Rabbits, anaesthetized with chlorpromazine (25 mg kg⁻¹) followed by sodium pentobarbital (20 mg kg⁻¹), were implanted with cannulae (David Kopf) consisting of a twenty-two gauge needle mounted on stainless steel tube 4 mm in diameter with threads cut on outside and inside. The inside holds a set screw to retain a neoprene seal. The threads on the outside enable the cannula to be screwed directly to the skull. The cannula was further secured by acrylic powder mixed with methylmethacrylate solvent. One week after insertion of the cannula, the rabbit was used for the experiment. Test materials were injected using a Hamilton 50 μ l syringe with an attachment to deliver 1 μ l at a time. The rabbit's rectal and ear skin temperatures were taken with copper constantan thermocouples and recorded by a Honeywell recording potentiometer. The rabbit was placed in a restrainer for 30 min before injection.

Four materials were injected into the test rabbits. To determine if the cannula was correctly placed, 'Piromen' (Travenol Laboratories, Morton Grove, Illinois), a *Pseudomonas* polysaccharide complex pyrogen, was injected. If the rabbit showed a fever, approximately 1° C rise, within 1 h of receiving 3 μ l of 'Piromen' (50 μ g ml⁻¹) it was used in the experiment. Rabbits were injected with an injectable grade saline solution containing 1% benzyl alcohol (Lilly) to determine if the cannula was clear of bacterial pyrogen and if the injection itself was responsible for the fever. As a control on leukocytic malarial-independent pyrogen, non-infected red blood cells were also injected. Malarial parasites and blood cells were obtained from female ICR white mice. The malaria parasite *Plasmodium berghei* strain NK65DA was transferred by intraperitoneal injection.

The level of parasitaemia was monitored by microscopically examining blood samples stained with Giemsa blood stain.

The blood of both the control and the infected mice was prepared in the same way. The mouse was anaesthetized with ether and the thoracic cavity opened. Heparin solution (0.1 ml), made by adding 0.1 ml of heparin (1,000 USP units ml⁻¹) to 2 ml of saline, was injected intraventricularly. The right lung was then removed and the blood was taken from the thoracic cavity and placed with 0.5 ml of heparin solution. The blood was then centrifuged at 1,000g for 10 min. The plasma and buffy coat supernatant (leukocytes)⁷ were discarded. The

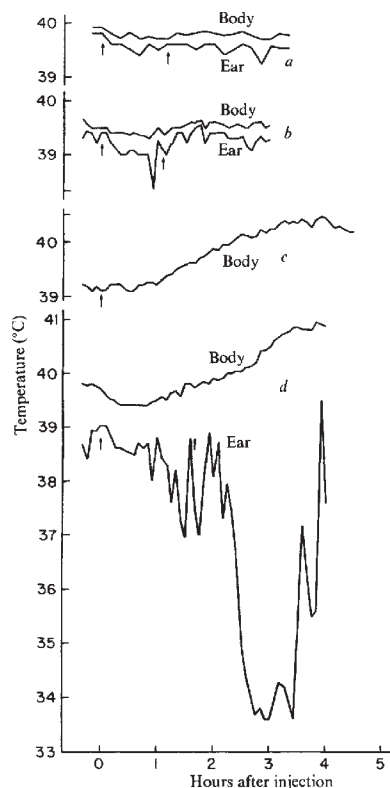


Fig. 1 Direct febrile response to malarial plasmodia. Changes in body temperature and ear temperature of a rabbit following microinjection into pre-optic/anterior hypothalamus. *a*, Saline control; *b*, non-infected RBC; *c*, 'Piromen'; *d*, infected RBC. Arrows indicate time of two injections of 2 μ l in *a*, *b*, and *d*, and of a single injection of 3 μ l in *c*. Saline and washed RBC from mice caused no changes in temperature (*a*, *b*). Bacterial pyrogen, 'Piromen', caused elevation of body temperature to febrile level more than 1 h after injection (*c*). Injection of malarially infected mouse RBC (*d*) causes elevation of body temperature over 1 h after injection. Elevation of body temperature is accompanied by marked decrease in ear temperature indicating vasoconstriction to conserve heat. Ear vessels dilate after 3.5 h as the body temperature stabilizes at febrile level.

packed red blood cells were resuspended with 2 ml of saline of the type mentioned before. The process was repeated four times. The last time the packed red blood cells were suspended in only 0.5 ml of saline and used for injection. The red blood cells were kept in an ice bath at all times except during centrifugation.

Both body temperature and ear skin temperature were within an expected normal range following the injection of saline into the pre-optic region of the rabbit (Fig. 1*a*). Normal mouse red blood cells caused only small fluctuations in ear and rectal temperatures. When the rabbit received malarial infected mouse red blood cells, the rectal temperature rose 1.5° C, and the ear skin temperature fell below 34° C, presumably due to

vasoconstriction in the ear to conserve body heat. The same set of materials, but in varying order, were injected two or three times in each of three rabbits with the same result, no change in body or ear temperature on receiving repeated doses of saline and washed normal mouse RBC, but increased body temperatures of 1.5 to 3° C and reduced ear temperature on receiving infected mouse blood cells or 'Piromen'.

Our results indicate that the presence of the malarial parasite is necessary for fever. The parasite may produce a pyrogen or induce the red blood cell to produce pyrogen. The malarial pyrogen differs from leukocytic pyrogen because the latency of the induced malarial fever is more than 1 h but the latency of the fever induced by a leukocytic pyrogen is about 10 min⁸. The timing and magnitude of the fever from malarial pyrogen resemble the response to the bacterial pyrogen, 'Piromen'. The malarial parasite, however, is a protozoan and eukaryotic and it lacks a cell wall, which is involved in production of bacterial pyrogen⁸. The chemical nature of the malarial pyrogen is unknown, but may differ from the classical division of natural pyrogens into leukocytic or bacterial.

Our studies do not distinguish whether natural malarial fever resulting from lysed red blood cells and plasmodium in the blood activating a leukocytic pyrogen⁹ or a pyrogen produced directly by the parasite is responsible for the fever. In the latter case, malarial fever might differ from the bacterially induced fever. Further characterization of the pyrogen associated with malarially infected red blood cells may yield a product useful for the study of temperature regulation and mechanisms of fever, as well as suggest new clinical procedures for dealing with malarial fever.

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Kidney Graft Survival and Matching for HL-A and ABO Antigens

THIS preliminary report considers 126 kidney transplants from cadaver donors performed in one centre over a period of 6 yr. All were first grafts. Previous sensitisation to human leukocyte antigens (HL-A) had been kept to a minimum by avoidance of transfusion where possible.

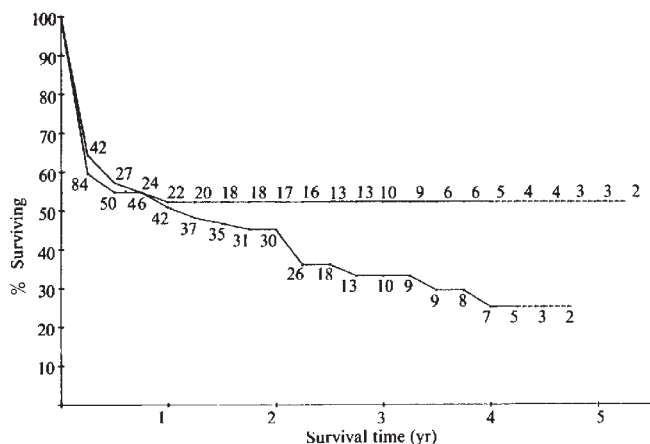


Fig. 1 Actuarial curves of group O and non group O kidney recipients showing total graft failure rate.

Eight patients had weak lymphocytotoxic antibody induced by pregnancy or transfusion before transplantation.

The criterion used for a successful graft at any one time was a kidney supporting life. Rejection was assessed histologically on allograft kidneys, removed surgically or at autopsy, without knowledge of the degree of matching.

Actuarial curves¹ were computed for three monthly periods using rejection as the criterion of failure. Those kidneys or patients that were lost for reasons other than rejection as assessed by clinical and laboratory examination (as, for example, haemorrhage, ureteric fistula or infection) were censored. Their survival was included in the actuarial curves while they were functioning, but their failure did not contribute to the percentage failure due to rejection. The numbers on the curves represent the number of individuals entering the previous three monthly period.

The HL-A antigens of donors and recipients were assessed at the time of transplantation, and also retrospectively on lymphocytes which had been stored in liquid nitrogen. A panel of well authenticated "monospecific" antisera was used. Many antisera were derived from the IVth International Histocompatibility Workshop. Specificities tested for were: HL-A1, HL-A2, HL-A3, HL-A9, HL-A10, HL-A11, W19, W28 HL-A5, HL-A7, HL-A8, HL-A12, HL-A13, W5, W10, W14, W15, W16, W17, W18, W21, W22, W27. For all except W16 and W21, several sera were used to determine each specificity.

In our study the basic assumptions underlying transplantation practice have been questioned. It is usually accepted that the rules applicable to blood transfusion in relation to the ABO groups also apply to transplantation of the kidney, namely when these rules are contravened, immediate (hyperacute) rejection usually supervenes. It seemed worthwhile, however, to examine whether those ABO categories, regarded as compatible for blood transfusion purposes, also behaved as compatible in organ transplantation. The O allele is believed to produce no antigen or erythrocytes, but it might form an immunogen on tissue cells.

The actuarial curves for the survival of group O recipients (who received kidneys only from group O donors) and non group O recipients of group A, B or AB (who received kidneys from ABO compatible donors) were compared for all graft failures and for failure attributable to rejection (Figs 1 and 2). The survival of group O recipients was better than that for non group O recipients. Rejection failure and survival, when assessed at specific periods, were not significantly different between the two groups. Using the regression method for life tables², however, which assesses differences between the groups over the whole time scale, the log-likelihood ratio is 1.99 ($P < 0.05$).

This suggests that the difference between the survival of group O and non group O recipients is real. The relatively

small number of grafts makes statistical significance difficult to obtain, but a previous series of forty-three grafts performed before HL-A typing was available shows the same trend of superior long term survival of group O recipients. The preferential survival of group O recipients, if real, might be due either to lower immunocompetence of group O individuals to antigens effective in eliciting graft rejection, or to an adverse effect of an incompatibility of the ABO system or an antigen linked with it. The incompatibility, for example, might conceivably be "antigen O" itself, and so a better survival might be expected in the category "donor A to recipient A" than in category "donor O to recipient A". Actuarial curves showed a slightly better survival for the donor A to recipient A group but this was not statistically significant.

We attempted to determine if group O individuals were less capable of responding to HL-A antigens than individuals of other groups. The ABO groups of ninety-four multiparous women who had developed cytotoxic antibody showed no disturbance from expectation. It seems therefore, that antibody produced to HL-A antigens by this immunisation route is not less in group O than in individuals of other groups.

In initial studies with the whole data on first grafts from cadaver donors, the influence of compatibility of the HL-A antigens seemed dubious. There seemed to be an increased survival rate for HL-A identity, but with the small numbers available this did not reach statistical significance.

In group O recipients there were no instances of failure due to rejection, after 9 months, and incompatibilities of HL-A antigens of either the first or second segregant series seemed to have no significant effect on survival.

In non group O recipients there was no significant relationship between rejection and incompatibilities at the first segregant series. A significant relationship did, however, exist between the number of incompatibilities at the second segregant series and rejection in this group (Fig. 3). Grafts having no incompatibilities at the second segregant series survive best, those having two incompatibilities the worst, and those with one incompatibility are intermediate ($P < 0.05$, using the regression method). This reinforces the observation³ that compatibility of the second segregant series is more important than of the first.

Our data suggest that for first grafts, loss of allografts by rejection is less in group O than in other groups, and that for the non group O recipients, matching at the second segregant series produces improved survival. Work in progress indicates that a different picture is presented by second grafts. If confirmed, these trends could make organ donor-recipient matching easier.

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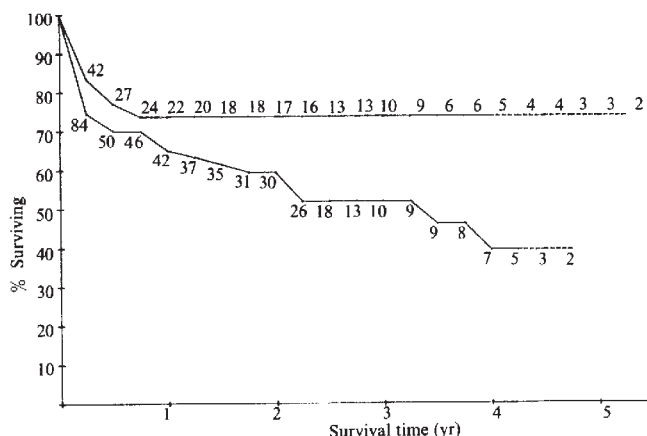


Fig. 2 Actuarial curves of group O and non group O kidney recipients showing failure rates due to rejection ($P < 0.05$).

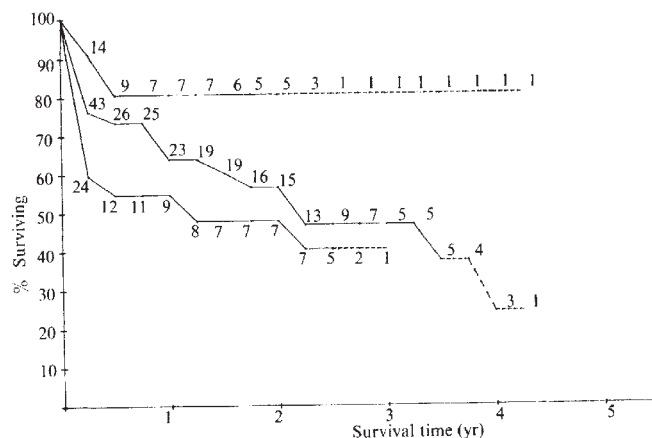


Fig. 3 Actuarial curves of non group O kidney recipients (that is, groups A, B or AB) showing failure rates due to rejection in recipients receiving kidneys with 0, 1, or 2 HL-A incompatibilities at the second segregant series.

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Effect of Sodium Fluoride on Regeneration in the Rabbit Ear

THE unique example of adult mammalian tissue regeneration seen in the rabbit's ear after removal of 1 cm² of its whole thickness¹ has been used to investigate the effects of procedures such as the administration of steroids on this regeneration²⁻⁵.

Sodium fluoride is known to be an enzyme inhibitor⁶⁻⁸ and apparently interferes with carbohydrate metabolism^{9,10} and inhibits cell growth¹¹. The effects of sodium fluoride on regeneration in the rabbit's ear could not, however, be anticipated from the results of these mainly *in vitro* studies. There is contradictory evidence regarding the effects of sodium fluoride on the inflammatory process. It has been suggested that this process, if occurring, is enhanced^{12,13} or reduced¹⁴. The inflammatory process may be an essential precursor to regeneration by the release of a substance or substances necessary for initiating regeneration.

Three groups of mature male rabbits were used: eighteen controls which received London tap water containing 0.3 p.p.m. sodium fluoride; a group of six which received drinking water containing 150 p.p.m. sodium fluoride; and a group of five which received drinking water containing 10 p.p.m. sodium fluoride. All rabbits were anaesthetised with intravenous pentobarbitone, and a hole 1 cm² in area was punched through the whole thickness of each ear with a special punch. Photographs of the ventral surfaces of the ears immediately after the operation (0 d) and at intervals of 7 d up to 49 d were taken with a cm ruler placed alongside the defect. The growth of the regenerated tissue was measured in terms of surface area using the method previously described¹⁵. The regenerated tissue was expressed as a percentage of the initial defect. Any reduction in the size of the hole due to contracture was excluded.

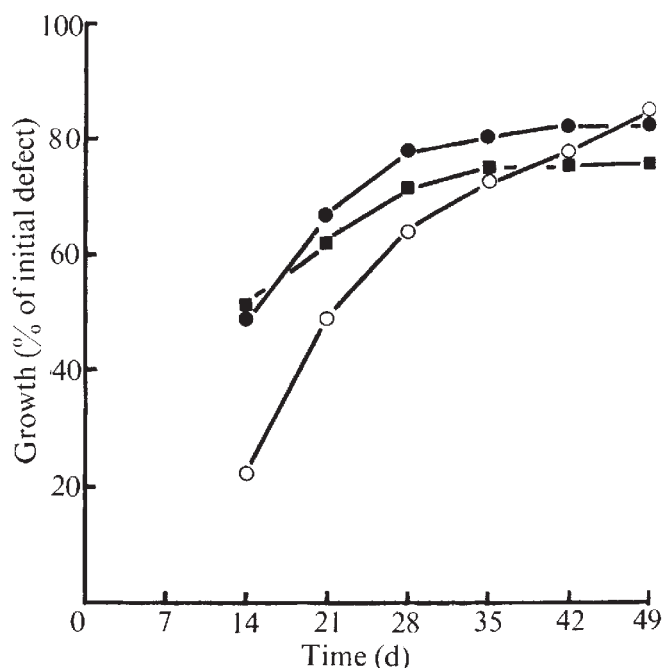


Fig. 1 Comparison of rates of growth at 7 d intervals: ○—○, controls; ■—■, rabbits receiving drinking water containing 10 p.p.m. sodium fluoride; ●—●, rabbits receiving drinking water containing 150 p.p.m. sodium fluoride.

Figure 1 shows the means of the new growth of tissue as a percentage of the original defect in the three groups at 14, 21, 28, 35, 42 and 49 d and Table 1 gives the means and standard errors of the means of the new growth at the same intervals. At 14 d there was a highly significant increase in regeneration in the two groups receiving sodium fluoride as compared with the controls at 14 d ($P < 0.005$). At 21 d the differences were still significant (for the group receiving 150 p.p.m. sodium fluoride $P = 0.001$ to 0.005 and for the group receiving 10 p.p.m. $P = 0.01$ to 0.02). At 28 d the 150 p.p.m. group still showed a significant increase in regeneration compared with the controls ($P = 0.02$ to 0.025) but in the 10 p.p.m. group regeneration was not significantly different from the control group. At 35, 42 and 49 d there was no significant difference between the three groups. (The difference between the 10 p.p.m. group and the control at 49 d was not significant: $P > 0.05$.) In the controls at 49 d, contraction caused about 3% of the reduction in the size of the hole, but in the animals receiving sodium fluoride contraction contributed much more (about 20%) to the decrease in the size of the hole. It should be emphasised that the technique used in this experiment measured only regenerated tissue. Figure 2 illustrates the marked difference in the amount of regenerated tissue seen at 14 d in the control (a) compared with the regeneration in an animal drinking water containing 150 p.p.m. sodium fluoride (b).

These results indicate that sodium fluoride in the doses administered produces a marked acceleration in regeneration

Table 1 Areas of New Growth as Percentages of Initial Defect at Weekly Intervals

Group	No. of rabbits	Mean areas of new growth $\pm$ s.e.					
		Time after excision (d)					
		14	21	28	35	42	49
Controls	18	22.3 ± 3.5	49.1 ± 2.4	63.9 ± 2.8	72.8 ± 2.5	77.0 ± 2.8	85.2 ± 2.6
Sodium fluoride 150 p.p.m.	5	49.2 ± 4.7	67.4 ± 5.2	77.7 ± 4.7	80.6 ± 3.7	83.6 ± 3.8	83.7 ± 3.8
Sodium fluoride 10 p.p.m.	6	50.9 ± 5.1	62.3 ± 3.6	71.8 ± 2.3	74.2 ± 1.5	74.9 ± 1.6	75.3 ± 1.8

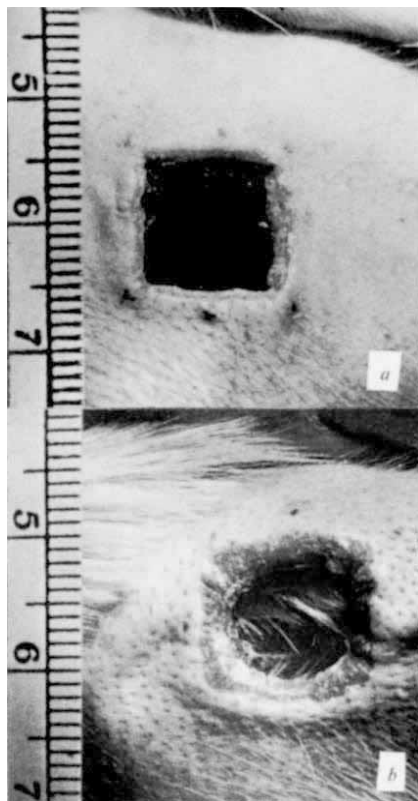


Fig. 2 *a*, Regeneration in ear of control rabbit at 14 d. *b*, Regeneration in ear of rabbit at 14 d after drinking water containing 150 p.p.m. sodium fluoride.

during the first three or four weeks following the whole thickness removal of 1 cm² of the rabbit's ear. It is difficult to state how sodium fluoride affects the process of regeneration since the factor or factors stimulating regeneration are not known. Present investigations suggest that at least four enzymes (acid phosphatase, alkaline phosphatase, non-specific esterase and cytochrome oxidase) are not reduced in the region of the blastema although these enzymes are reported to be inhibited by fluoride^{6-8,16}. Regeneration may be stimulated by a wound hormone produced by cell damage following injury and sodium fluoride may increase this early cell damage with the result that the stimulus to regeneration is enhanced. Preliminary investigations suggest that this is the case since there appears to be an increase in the acid phosphatase present 2 d after operation in the fluoride-treated animals as compared with control animals at the same stage. This could be due to increased lysosomal breakdown stimulated by the presence of sodium fluoride.

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Isomerisation of the Visual Chromophore All-trans to 11-cis Retinal

It is well known¹ that the only action of light in vision is the photoisomerisation of the 11-cis retinylidene (derived from vitamin A aldehyde) prosthetic or chromophoric group of rhodopsin, from the 11-cis to the all-trans configuration. After several poorly understood intermediate steps rhodopsin is bleached to opsin, retinal being set free in the all-trans form. Two paths are now possible for the all-trans retinal: either isomerisation to the 11-cis configuration and joining with opsin to form rhodopsin (the situation here, however, is not clear and is likely to be rather complex^{2,3}), or reduction to retinol (vitamin A) and, after esterification, storage as the 11-cis ester⁴.

Since there are six known isomers of retinal, namely all-trans, 9-cis, 11-cis, 13-cis, 9-13 dicis, and 11-13 dicis, the specific isomerization of the all-trans to the 11-cis configuration and the large amounts of the 11-cis form present in the eye⁴ have long been an enigma. Indeed Wald¹ has asked "why should three phyla independently have selected for vision such a fundamentally improbably, hindered *cis* configuration of retinal (as the 11-cis configuration)?"

In the course of developing a method for simulating *ab initio* molecular-orbital calculations⁵ we have studied all-trans retinal using the results of *ab initio* calculations on relatively large "pattern" molecules including 1,1,3-trimethyl cyclohex-2-ene and crotonaldehyde. The geometry employed is that given by Hamanaka *et al.*⁶ with the modification that all carbon-hydrogen bond distances are set equal to 1.08 Å and the side chain is idealised (that is, the carbon-carbon single and double bond distances are set equal to 1.50 Å and 1.34 Å respectively). This modification allows simulation of the side chain from a small number of pattern molecules. We have found that the results of the simulated *ab initio* molecular orbital (SAMO) method are of comparable accuracy to those that would be obtained by using the much more expensive complete *ab initio* approach. Although the full results of our SAMO calculation on retinal will be published elsewhere we report here some interesting features which may help in understanding the above enigma.

In Fig. 1 we show the numbering scheme used for all-trans retinal. We shall label the occupied π orbitals, in decreasing order of energy, as $\pi_1, \pi_2, \dots, \pi_6$ and the virtual (unoccupied) π orbitals, in increasing order of energy, as $\pi_1^*, \pi_2^*, \dots, \pi_6^*$.

The total overlap populations of all-trans retinal show the π bonding character to be weakest in the region of the C₁₁-C₁₂ bond. From this, in the ground state one would

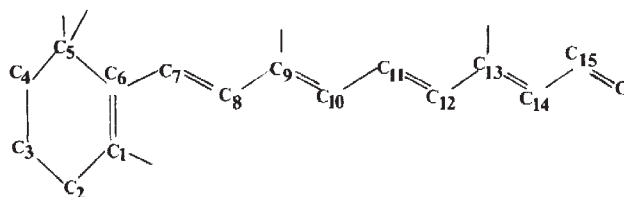


Fig. 1

Table 1 Total Overlap Populations for Various Configurations of All-trans Retinal

Configuration	Bonds			Energy of transition (a.u.)
	C ₉ -C ₁₀	C ₁₁ -C ₁₂	C ₁₃ -C ₁₄	
Ground state	0.23488	0.23180	0.23545	
$\pi_1-\pi_1^*$	0.06285	0.07087	0.12687	0.35107
$\pi_2-\pi_1^*$	0.14382	0.10610	0.10672	0.40001

expect isomerisation to be favoured at this position. This feature, although it has been observed from the much less accurate Pariser-Par-Pople (π electron only) calculation of Pullman and Pullman⁷, has now been confirmed by our more accurate all electron method.

Although the SAMO method produces both occupied and virtual orbital energies for the ground state, since it does not explicitly use electron repulsion integrals, we have been unable to perform configuration interaction calculations. We have, however, been able to estimate the total overlap populations for various configurations. The lowest energy $\pi-\pi^*$ triplet state will best be described by a mixing (configuration interaction) of a number of configurations each formed by the promotion of an electron from an occupied π orbital to a virtual π^* orbital. The lowest-lying virtual π^* orbital, namely, π_1^* , is very much lower (having an energy of 0.02689 a.u.) than the other π^* orbitals. The most important of the configurations describing the $\pi-\pi^*$ excited state will be the $\pi_1-\pi_1^*$ configuration. The next most important will be the $\pi_2-\pi_1^*$. The total orbital populations for these configurations in the ground state are shown in Table 1. Configurations arising from the promotion of an electron from π_1 or π_2 to π_2^* are likely to make very small contributions to the $\pi-\pi^*$ excited state, because of, first, the energy difference and, second, the symmetry of the orbitals locally within the chain. The π orbital population in the $\pi_1-\pi_1^*$ configuration shows that bonds C₉-C₁₀ and C₁₁-C₁₂ are weaker than all other double bonds, the C₉-C₁₀ bond being marginally weaker than the C₁₁-C₁₂ bond. In the $\pi_2-\pi_1^*$ configuration, however, the C₁₁-C₁₂ bond is substantially weaker than the C₉-C₁₀ bond. Thus although we were unable to estimate the amount of mixing of these two configurations, it would seem feasible that there is a sufficient contribution from the $\pi_2-\pi_1^*$ configuration to make the C₁₁-C₁₂ bond the weakest double bond in the excited triplet state.

This leads us to suggest that the preferred isomerisation about the C₁₁-C₁₂ bond is due to the weak nature of this bond in the excited triplet state and that this would be established by a detailed all electron *ab initio* configuration interaction calculation. Such a calculation is economically impossible at present so that the conclusions from our simulated *ab initio* study are of some interest.

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Formation and Breakdown of Aggregations of the Crown-of-Thorns Starfish, *Acanthaster planci* (L.)

IN spite of its notoriety, population densities of *Acanthaster planci* (L.) on most flourishing reefs in the Indo-West Pacific are low. It is possible, however, to find reefs in the region which support aggregations of hundreds or thousands of this starfish¹⁻⁸. In the Sudanese Red Sea we usually found *A. planci* densities of 5 to 20 per km of reef face, but on four reefs aggregations of several hundreds within a few hundred metres were studied⁹. These starfish were not evenly distributed but were usually separated into small subgroups of up to fifteen animals. Such subgroups tended to be associated with favoured species of corals, such as *Goniastrea*, *Turbinaria*, *Montipora* and tabular *Acropora* spp., whose shapes allowed several individuals to feed together and provided shelter from daylight.

We observed that *A. planci* leaving a completely eaten-out *Acropora* table often moved to other tables on which starfish were already feeding, rather than to tables which were as yet untouched. Such observations suggested some form of attraction between individuals. This appeared to influence the aggregation as a whole and our marking studies showed that its integrity was maintained over 3 months, implying some cohesive force. Nocturnal observations were made on the behaviour of two subgroups using tags to identify individuals. In Fig. 1 the mean distances between all the marked starfishes (the proximity index) are plotted against time. This index reveals that within the subgroups, starfishes moved closer together after nightfall once feeding had begun, and it suggests that feeding individuals exert a chemo-attractive influence on hungry starfishes nearby.

To extend our investigations into this possible chemo-attraction mechanism we sought to demonstrate that hungry starfish would select between two alternative food sources provided for them. Preliminary experiments using two large wire mesh Y cages arranged on the seabed were carried out. These allowed hungry starfish to move freely from the shelter of dead corals provided in the stem of the cages, and to choose

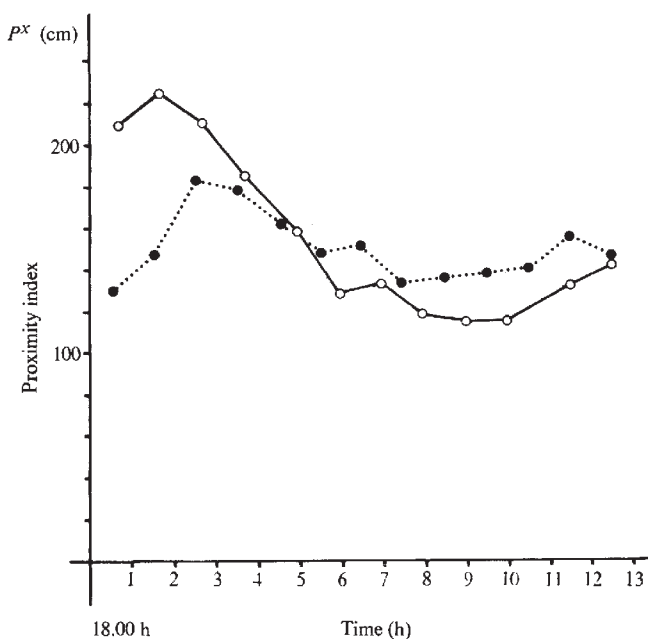


Fig. 1 Variation in the mean inter-starfish distance (proximity index $P^x = \sum |x^i - x^j| / [n(n-1)]$; $i \neq j$, $i, j = 1, 2, \dots, n$) with time for two groups (○ and ●) of *A. planci* studied at night. The index for both groups decreases after 2 h when some of the starfish had started feeding. The observation areas were 15 m² and there were eight starfish in each group.

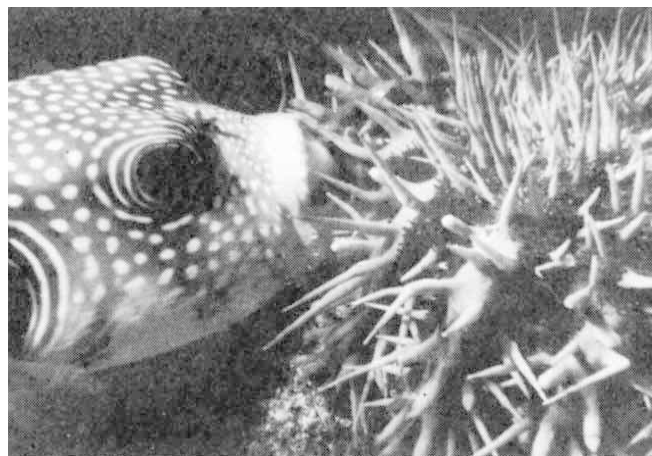


Fig. 2 *Arothron hispidus* (L.) feeding on *Acanthaster planci*.

between potential sources of attractant, including other *A. planci* already feeding, located in the arms of the Y. These experiments, which were run at night, indicated an order of preference of attractants in which feeding *A. planci* were more effective than live coral alone, which in turn was more attractive than dead coral.

Because of the mesh structure of the cages, and the possible effects of currents of seawater, it was decided to abandon these tests in favour of a Y maze which took the form of a large aquarium. The base of the Y stem was relatively deep (0.5 m) and allowed seven or more experimental starfishes to hide under shelter during daylight. Seawater was pumped into the arms of the Y maze fresh from the sea. This caused equal currents to pass along each arm, over the attractant sources provided there, towards the base where it flowed out of the system. At dusk the experimental animals became active, and on moving could choose to enter either of the arms. The positions of the alternative attractant sources were reversed from night to night in each experimental series to rule out any inbuilt bias of the maze.

To date, this apparatus has yielded the following results. First, *Acropora* sp. against no attractant: thirty-nine choices to fifteen ($\chi^2 = 10.67$, $P < 0.05$). Second, *A. planci* feeding on *Acropora* sp. against no attractant: thirty-one choices to eleven ($\chi^2 = 9.52$, $P < 0.05$). Third, *A. planci* feeding on *Fungia* sp. against intact *Fungia*: thirty-four choices to fourteen ($\chi^2 = 8.33$, $P < 0.05$).

The first result indicates that *A. planci* navigates towards its prey by chemosensory means like other asteroids (R. J. M., unpublished, and refs 10 and 11) and the second and third show that the feeding activity of *A. planci* on coral releases material which, in favourable conditions, will stimulate other starfish towards its source. This we regard as evidence for our chemo-attraction theory.

Our observations on the chemo-attraction effect in the feeding starfish have led us to propose the following model for aggregation formation. The distribution of low-density populations may be explained as a function of recruitment from dispersed settling larval starfish and the independent migration of solitary adults. An orthokinetic effect³ may serve to influence adult movements, as their rate of progress over the reefs is almost certainly affected by the ease with which they can find adequate food and shelter from daylight.

We found that starfishes preferred areas which were not too exposed to turbulence, not sandy and in which there was an adequate supply of preferred food corals. Within particularly favoured areas the effects of this mechanism, probably enhanced by chemotaxis towards preferred corals, may be sufficient to lead to slight local aggregations. At this state a critical effect seems to operate, for when the starfish come close enough for them to perceive the chemo-attractants described above, the

subgroups of the aggregation form. Local environmental changes may also occur which enhance the development of these subgroups, which we found to develop at densities of thirty to fifty individuals per km of reef face.

We also believe that the activities of an aggregation may influence larval survival and recruitment of juveniles to the population with important consequences in the long term. The increased proximity of adult starfishes may enhance the chances of fertilisation, especially if synchronous spawning takes place as has been described for other echinoderms¹²⁻¹⁴. Further, our group has recently demonstrated that the larvae of *A. planci* settle preferentially among clean calices of some species of coral and also that living corals prey on settling larvae (M. F. Barker, unpublished). Thus, as Chesher suggested¹⁵, the destructive influence of aggregated adult starfishes will allow an increase in the successful recruitment rate of the juveniles, as in these circumstances larval settlement will not be accompanied by such high mortality. This consideration indicates that the population density of *A. planci* at which aggregation into groups begins may constitute a threshold beyond which a population explosion is likely to occur. The populations of *A. planci* may therefore be particularly sensitive to small changes of significant environmental factors which could result in densities in the region of this threshold; whether as a result of migration and convergence of adults, or due to an initial small increase in juvenile recruitment. Such small changes may be natural or man made.

Our research has indicated that the predatory behaviour of certain species of fish may be important in controlling the densities of adult *A. planci* in the central Red Sea. By continually working under water on one particular reef, many fish became accustomed to the presence of our divers so that their behaviour could be freely observed. Thus, two previously unreported predators of *A. planci* were discovered: the puffer fish *Arothron hispidus* (L.) (see Fig. 2) and the trigger fish *Balistoides viridescens* (Bloch). Both these fish attacked in a characteristic fashion, and the puffer fish left readily recognisable remains in the form of a circlet of detached starfish rays. The trigger fish *Pseudobalistes flavimarginatus* (Rupp), previously reported as a predator of the crown-of-thorns¹⁶, was also seen attacking it in the Red Sea. If these predators respond to an initial increase in the starfish population by feeding more frequently on *A. planci*, they may be capable of an early breakup of an aggregation. Thus if the formation of small aggregations of the type we have been studying is a critical stage in population explosion, these fish predators may be a particularly significant factor in controlling rapid increases in the sizes of *A. planci* populations.

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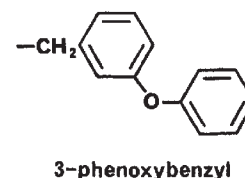
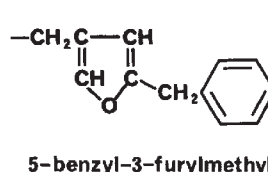
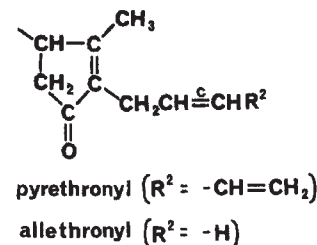
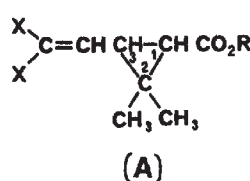
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A Photostable Pyrethroid

THE natural pyrethrins (see refs 1 and 2 for references) (for example, pyrethrin I (1)) and related synthetic insecticides^a (allethrin (2), bioallethrin (3), resmethrin (4), bioresmethrin (5), Cismethrin (6) and the ethanochrysanthemate (7)) are unstable in air and light (see refs 1 and 2 for references, and ref. 4). This property restricts their use, particularly against pests of agricultural crops, in spite of other favourable characteristics (see refs 1-3 for references) (outstanding potency against many insect species, rapid action, and low mammalian toxicity^b). We describe here new synthetic esters, ten to one hundred times more stable in light than previous pyrethroids, yet as active against insects as bioresmethrin (5) and with low mammalian toxicity.

A recent modification^c of the acid component of bioresmethrin (5)^{7,8} gave a compound (8), 5-benzyl-3-furylmethyl (1R, trans)-2,2-dimethyl-3-(2,2-dichlorovinyl) cyclopropane carboxylate,



more potent than most known insecticides (LD50 0.1-0.3 mg kg⁻¹ to houseflies and mustard beetles). Chemical considerations^a and spectroscopic evidence indicated that the furan ring in esters of 5-benzyl-3-furylmethyl alcohol was a probable site for photosensitized attack by oxygen (now confirmed for resmethrin¹⁵). Therefore we examined esters of the modified acid with other alcohols. Other esters of potentially photostable alcohols (for example, 2,4-dimethyl benzyl¹⁰, 2,4,6-trimethylbenzyl¹¹, 6-chloropiperonyl¹⁰ and 3-benzylbenzyl^{8,12} even with the most effective acids such as chrysanthemic and ethanochrysanthemic¹³ have been insufficiently active for practical application. In contrast, the ester (9) of 3-phenoxybenzyl alcohol^{8,14} with the 2,2-dichlorovinyl acid was more active against insects than would have been predicted. It was as potent, for example, as bioresmethrin (5) to houseflies and over 2.5 times more potent to mustard beetles; the corresponding ($\pm$)-cis,trans esters (11) and (4) had comparable differences in activity. The ester (11) was also three to four times as effective as the corresponding chrysanthemate to *Anopheles stephensi* females and *Glossina austeni* (mixed sexes) (results communicated by F. Barlow).

To determine the stability in light of the new esters, deposits (0.2 mg cm⁻²) were exposed to daylight near a window indoors. In these conditions 50% (determined by gas chromatography)

Table 1 Toxicity of Pyrethrins to Insects and Mammals

Compound	Name or designation	X in (A)	Stereochemistry	R in (A)	Relative toxicities to:		Approximate lethal dose to rats (mg kg ⁻¹)	
					Houseflies (<i>Musca domestica</i> L.)	Mustard beetles (<i>Phaedon cochleariae</i> Fab.)	Oral	Intravenous
(1)	Pyrethrin I	CH ₃	1R,3R	(+)-pyrethronyl (S at C-4)	2	160	260-420**	5
(2)	Allethrin	CH ₃	Mixture†	(±)-allethronyl	3	1	—	—
(3)	Bioallethrin	CH ₃	1R,3R	(±)-allethronyl	10	4	1,000	4
(4)	Resmethrin	CH ₃	Mixture†	5-benzyl-3-furyl-methyl	42	37	>3,000	160
(5)	Bioresmethrin	CH ₃	1R,3R	5-benzyl-3-furyl-methyl	100	100	>8,000	340
(6)	Cismethrin*	CH ₃	1R,3S	5-benzyl-3-furyl-methyl	41	64	100	6-7
(7)	"Ethanochrysanthemate" [†]	X-X= (CH ₂) ₄	1R,3R	5-benzyl-3-furyl-methyl	140	170	100††	5-10
(8)	NRDC 134	Cl	1R,3R	5-benzyl-3-furyl-methyl	220	260	>400††	26-33††
(9)	NRDC 147	Cl	1R,3R	3-phenoxybenzyl	100	270	—	—
(10)	NRDC 146	Cl	1R,3R+1S,3S	3-phenoxybenzyl	42	140	>2,000	>270
(11)	NRDC 143	Cl	Mixture§	3-phenoxybenzyl	60	120	1,500§§	>270§§

* NRDC 119, Decis = RU 12063.

† K-othrin = RU 11679.

‡ 70% (±)-trans, 30% (±)-cis.

§ 80% (±)-trans, 20% (±)-cis.

|| Determined by comparison of LD50 values from topical application of 1 µl drops of acetone solutions to adults.

¶ For solutions of the compounds in glycerol formal except where stated otherwise. Results communicated by Mr R. D. Verschoyle (compounds (6) and (8)-(11)) or taken from ref. 4.

** Administered in dimethyl sulphoxide.

†† Administered in olive oil. Result communicated by Roussel-Uclaf.

‡‡ For the (±)-trans compound (NRDC 140).

§§ Administered in arachis oil.

of esters (4–7) of 5-benzyl-3-furylmethyl alcohol was lost in 4–6 h whereas the 3-phenoxybenzyl ester (11) lasted longer than 3 weeks. Similar deposits were also exposed out of doors under quartz plates which shielded them from rain and wind but admitted ultraviolet and visible light. In these accelerated tests, where films attained up to 50°C in bright weather, bioresmethrin (5) lasted 1–2 h, compared with about 4 d for the 3-phenoxybenzyl ester (11). Bioassays of deposits with *Drosophila melanogaster* also indicated comparable persistence of insecticidal activity, showing that the photodecomposition products were not toxic to insects in these conditions.

Although such greater stability should extend scope for pest control, persistence of the new esters is moderate and therefore the advantage of freedom from toxic residues should be retained. In addition, their mammalian toxicity is low (see Table 1); for instance, the (±)-*cis,trans*-dichlorovinyl ester (11) containing about 20% of *cis* component (easily obtained by heating the mixed acid chlorides before esterification), like resmethrin (4), had lower oral and intravenous toxicities to rats than pyrethrin I (1) or bioallethrin (3).

Work is in progress to identify the photodecomposition products and mammalian metabolites of the dichlorovinyl esters and to establish the behaviour of the compounds under practical pest control conditions.

The new compounds are protected by UK Patent Applications Nos 30838/72 and 59184/72. We thank Dr J. M. Barnes and Mr R. D. Verschoyle for communicating mammalian toxicity data, Mr F. Barlow for results with *Glossina austeni* and *Anopheles stephensi*, and the National Research Development Corporation for financial support.

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In the article "Potent Pyrethroid Insecticides from Modified Cyclopropane Acids" by M. Elliott *et al.* (*Nature*, **244**, 456; 1973) two references should be added:

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and so refs 10 and 11 on Fig. 2 should be changed to 14 and 15 respectively.

Erratum

In the article "Transcellular Strands of Cytoplasm in Sieve Tubes of Squash" by Robert Thaine and Margaret E. De Maria (*Nature*, **245**, 161; 1973) Fig. 2 was reproduced on too small a scale for the salient features to be clearly visible. It is reproduced again here.

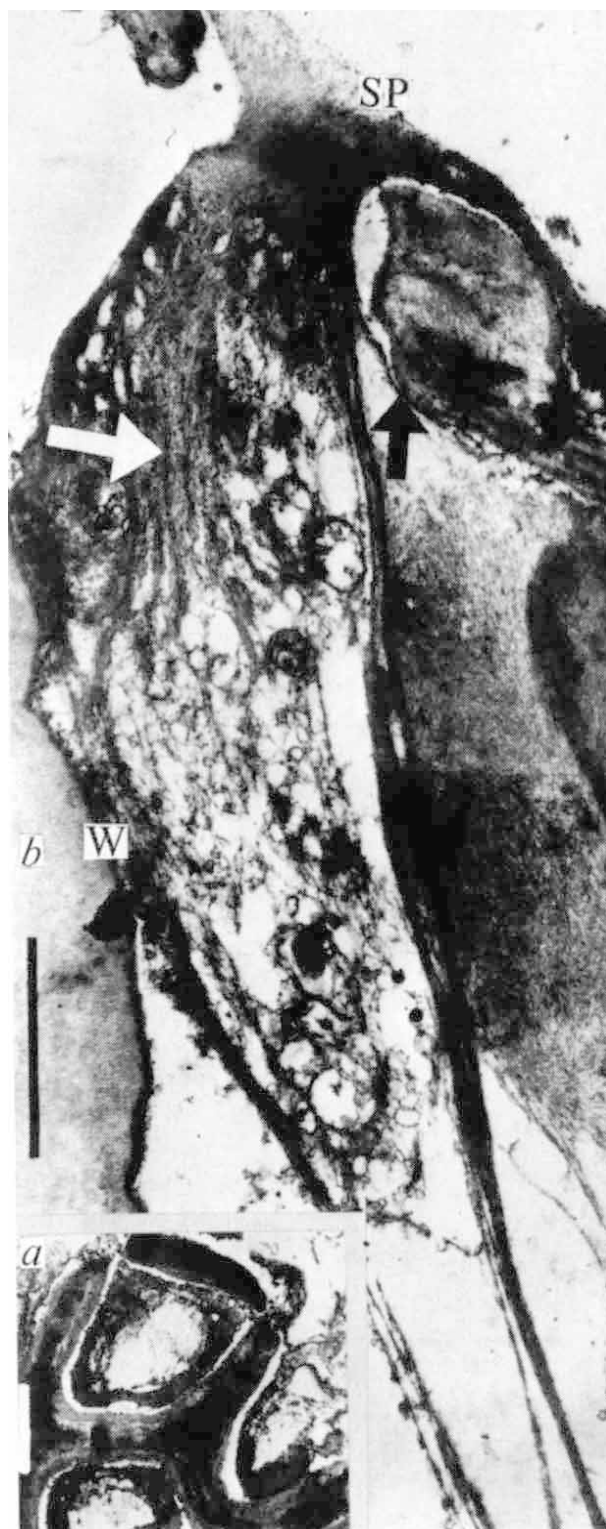


Fig. 2 *a*, An electron micrograph from a transverse section of a sieve plate in which pores have a tubular lining of densely stained material. The bar represents 2 μm. *b*, An electron micrograph from a longitudinal section showing a strand extending from a sieve pore (SP) into the sieve element. W, Sieve tube wall. Black arrow, plasmalemma; white arrow, a group of parallel structures which can be followed from the constricted end of the broken strand (see text). The bar represents 2 μm.

BOOK REVIEWS

The Art of Science

From Stonehenge to Modern Cosmology. By Fred Hoyle. Pp. 96. (W. H. Freeman: San Francisco and Reading, March 1973.) £2.10.

AN art historian from Vienna once gave me his personal view of contemporary art—and here I extrapolate freely from memory—that the art of the times is science. He held that some day we will look back on our present science as an essentially religious enthusiasm and the cyclotrons and telescopes that did this science as representative works of folk art.

It is fascinating to read, then, Fred Hoyle's absorbing analysis of Stonehenge as an ancient astronomical observatory. It feels right that the stone age builders of this monument, inextricably close to their environment, should be strongly aware of the eclipses of the Sun and the Moon. It is an intellectual pleasure to follow Hoyle's reconstruction of how the post holes and sighting lines enabled eclipses to be predicted. It was an elevating thought for me to consider that these ancients, with their stone age orrery, had probably a simple, accurate grasp of the spatial relationships of their nearby, celestial neighbours.

But then the question arises: Why did the functions of Stonehenge I decay? Did the early Britons find the smelting of metals more useful than the predicting of eclipses? It now becomes clear that of the four short lectures that comprise this ninety-three-page book, the one on Stonehenge should have come first. The one that is actually first, "Science and Society in Modern Times", should have come second because it raises the question of what makes a society rise and decline. Hoyle notes a tension between science and society. He notes the obvious fact that science, technology and its long range consequences are not deeply understood by many politicians. Hence science is often applied destructively rather than constructively to society. But he does not discuss what is, to me, the more crucial point; that is, the effect of society on scientists. Surely scientists reflect the competitiveness and insecurity of their milieu. They manoeuvre for pre-eminence, squabble over marginal doctrinal issues, and only unite to suppress the threat of new ideas. The real tragedy—beyond the fact that science is not much fun under these conditions—is that science is then misapplied by shortsighted or unscrupulous people from other sectors of society. The scientists have no effective

chance of ensuring that their product is used constructively because they are totally disunited.

If we were ever to live in a healthy, friendly, intelligent world, perhaps the last two chapters in this book would furnish us a glimpse of the kind of activities we would be engaged in. The broadest possible questions are addressed—like the distances of quasars and galaxies, the nature of mass, the phenomenon of seafloor spreading in the Earth. Hoyle faces the hard observational evidence for discrepancies in the conventional cosmology and tries to gain understanding by connecting inferences from many fields of astronomy, physics, and science. Needless to say, his suggestions are considered controversial. It is often said about someone who is obviously very bright, but clearly a non-conformist that, well, maybe he is incorrect but he is valuable because he stimulates thought. That is not quite the point. On many of the issues raised in this book there are right and wrong answers. At least there are some answers that are a good deal more correct than others. About only one thing can we be fairly certain and that is that the *status quo* is incomplete. If we have much of a future, our current understanding of reality will eventually appear extremely primitive. Which are now the most correct answers?

If we progress in our understanding it will have to be through an unprejudiced awareness of our environment. Perhaps the builders of Stonehenge I had such a harmonious attitude toward their surroundings. Perhaps some day we will pursue fundamental questions such as what is the nature of the universe in a more leisurely and co-operative fashion, like an art. And perhaps that will be the most rigorous analysis of all.

HALTON ARP

Buffalo

The Time of the Buffalo. By Tom McHugh with the assistance of Victoria Hobson. Pp. xxiv + 339 + xi. (Alfred Knopf: New York, December 1972.) \$10.

THIS is a long, in parts detailed but for the most part readable book about both North American subspecies of the so-called buffalo—*Bison bison bison*, the plains buffalo, and *B. bison athabasca*, the wood buffalo. To a much lesser extent it is also about their ancestors and near relatives. It is not clear, however, what readership the author had in mind when writing it, although the somewhat vague, rather nostalgic title

seems to suggest that a wide market is envisaged. This lack of focus may be a consequence of the author's three professional interests: research zoologist with a special interest in animal ecology and wildlife management; author; and photographer for several of Walt Disney's True Life Adventure films, including *The Vanishing Prairie*. It is most certainly not a book for the anatomist, physiologist or palaeontologist and scholars interested in the historical aspects of the buffalo will find that it lacks the depth of Frank G. Roe's *The North American Buffalo: a Critical Study of the Species in the Wild State* (University of Toronto Press, Toronto, 1970).

Much of the book is concerned with the Indians' use of and attitude towards the buffalo at and immediately after the period of first white contacts but it is not a significant contribution to cultural anthropology. The author's real contribution is that, working as an animal ecologist, he has spent considerable time observing at close range the present-day herds in Yellowstone National Park, Wind Cave National Park, the Wichita Wildlife Refuge, the National Bison Range and the Crow Reservation. His objective in doing so was to try to answer some of the "legacy of questions" left by the "vast literature on the beast", not by a further re-sifting of the literature but by personal observations. How did hide-hunters manage to kill large numbers from a single group without frightening the survivors? Did the vast herds wander erratically or make precise annual migrations? Did vast numbers actually die in blizzards, drownings, quicksands and prairie fires? It is from his own observations that the author contributes most in attempting to answer questions like these and it is when he writes of these observations that he is at his most compelling.

Not surprisingly, in the light of the author's professional experience as a photographer, the book is illustrated with some excellent monochrome prints of buffalo in action: at birth and play; wallowing and fighting; and being lured into traps by the author's successful application of the techniques employed by Indian decoys. One would have welcomed more of these, even if it had been at the expense of some of the reproductions of nineteenth century etchings, paintings and photographs of buffalo hunts and hunting techniques. Four pages of maps are given pride of place at the front of the book. These are useful but the two showing the whole of North America lack clarity. There

is a nineteen-page bibliography and the book is generally well produced.

G. MALCOLM LEWIS

Industry and Education

The Universities and British Industry, 1850-1970. By Michael Sanderson. Pp. x+436. (Routledge and Kegan Paul: London, December 1972.) £6.50.

OVER the greater part of the nineteenth century the ancient universities of England were indifferent to industry and did nothing to encourage graduates to take up industrial careers. On the other hand, the provincial universities, founded after 1850, began as science colleges and were always sensitive to the needs of local industry; this was also true of most of the London colleges. By 1914 the universities, including the appropriately reformed Oxford and Cambridge, were able to make considerable technological contributions to the war effort, and they were able to repeat this service during the Second World War. Since 1945 there has been a proliferation of new universities and a great increase in the number of subjects available for study.

Dr Sanderson has chronicled these developments, which I take to be fairly well known, in some detail and he has confirmed most of the conclusions we usually draw from them. He discusses the English university institutions in relation to industry and he has chapters dealing with the Welsh colleges and (particularly interesting) the Scottish universities as well as with special topics such as commercial education and the woman graduate in industry and commerce. He has studied the main sources of support of the universities and has examined the distribution among faculties and subsequent careers of the students. He has sifted an immense amount of material. But there are so many universities and so great is the scope of industry that his work has certain obvious limitations. Apart from Belfast and Coleraine, the Irish universities are not mentioned although they were in the United Kingdom for more than half Dr Sanderson's period. American, French and German institutions come only very marginally into the story, while technical colleges are mentioned only when they become universities. Unsuccessful attempts to found universities are ignored: there is no mention of, for example, the interesting proposal, a hundred years ago, to found a Western University in the Vale of Neath.

Universities, however, do not exist in a social void. The development of British universities, and hence their value to industry, was necessarily restricted by the unsatisfactory state of

primary and secondary education before 1902, the inadequate scholarship ladder and the expense of attending a British compared with a German university. Dr Sanderson does not mention these aspects of the story and he does not comment on the long debate about the role of examinations in university education, to which was related the nineteenth century failure to develop research training. These factors account, in part, for the domination of British industry, and even of British scientific education, by Germans in the early 1900s; a domination that Dr Sanderson notes but does not analyse. Parenthetically, it is notable that the phenomenon was most conspicuous in manufacturing areas of "high technology", such as the Manchester region. The contemporary response included the heroic efforts of Sir Norman Lockyer and the establishment of the British Science Guild.

I am, therefore, puzzled by Dr Sanderson's conclusion that the best British universities had, by 1914, caught up with their German equivalents. Many novel features of British universities in the early years of this century had been explicitly copied from German practice: Imperial College was called the "London Charlottenburg". By 1914 the great majority of professors of chemistry in British universities had German PhD degrees. And, if the situation was by then so satisfactory why was it later thought desirable to introduce the German PhD system into this country?

In fact the tenor of Dr Sanderson's argument is that, generally speaking, we may congratulate ourselves on the way things have gone. Indeed he is almost complacent in his comment that we have avoided the "dualism" of universities on the German model, divided as they are between the traditional and the technological. Surely we are not called on to commiserate with, for example, the Swiss since Zürich is lumbered with both a traditional university and the Federal Institute of Technology?

In short it is not easy to grasp the point of this book. It brings to light no serious problems, hitherto unnoticed, and because of the enormous range of the subject matter it cannot deal in depth with specific issues. I assume therefore that Dr Sanderson intends it as a pilot study and will now go on to make detailed investigations of individual topics. Certainly one hopes that this is the intention; there are, after all, enough problems of modern and of historical importance to deserve the attention of a scholar like Dr Sanderson who is not afraid of hard work and who is capable of dealing with great quantities of intractable material.

The minor criticisms of this book must reflect the interests of the individual reviewer. It is clear to me that Dr Sanderson overlooks the sharp distinction that Whewell drew between "established" sciences (astronomy, mechanics) and "progressive" sciences (such as botany, zoology); it was the latter, Whewell argued, that should not be taught to undergraduates. He is also rather less than fair to Mark Pattison, who became a progressive in his later years and played a part in the "endowment of research" movement. As an adoptive Mancunian I object to Dr Sanderson's cavalier spelling of Fairbairn's name. I think he exaggerates, at the expense of Harwood and Bateman, the part played by Roscoe in the Thirlmere scheme. And, much more serious, he does not mention George Davis, the pioneer of chemical engineering as an academic subject (see his *Handbook of Chemical Engineering*, 1901). Finally, it should be pointed out that only a part of the Royal Navy Signals School, later the Admiralty Signals and Radar Establishment, was moved to Bristol University during the last war; other portions went to places like Haslemere and Witley.

D. S. L. CARDWELL

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Denington Estate,
WELLINGBOROUGH,
Northants.

Wealden Geology

Geology of the Country around Royal Tunbridge Wells. (Memoirs of the Geological Survey of Great Britain, England and Wales.) By C. R. Bristow and R. A. Bazley. With contributions by R. W. Gallois, E. R. Shephard-Thorn, R. Allender, R. Casey and H. C. Ivimey-Cook. Pp. x+161 (9 plates). (HMSO: London, November 1972.) £2.

THE memoir is an explanation of One-inch Geological Sheet 303, New Series, which was resurveyed in 1960–1965 and published in 1971. The area is centrally situated in the Wealden Basin, and at outcrop the solid rocks are almost all of Wealden age with a very small area of Purbeck strata in the extreme south-east. The memoir includes an account of the stratigraphical palaeontology of Jurassic strata penetrated by the Ashdown boreholes, one of which terminated in undated, pre-Jurassic sediments.

After an introductory section, the remaining seven chapters deal with structure, concealed formations, the Jurassic, the Cretaceous, the Pleistocene and Recent deposits, superficial structures and economic geology. Each chapter is composed of individual (initialled) paragraphs by the field surveyors and specialist contributors, which generally fit together well to give a thorough account of the area. An anomaly arises because of the unfortunate use of "Jurassic" and "Cretaceous" as chapter headings: the Purbeck Beds are discussed under the Jurassic despite the apparent acceptance of Casey's views on the age of these beds.

Of the eight chapters, that on the Cretaceous (Wealden Beds) occupies seventy-one out of the 123 relevant pages. There is a general account of the Hastings Beds Group, which is divided into five mappable formations (the term "formation" in parentheses). These represent the deposits of three major cyclothems of deltaic sedimentation. Ostracods provide the most useful zonal fossils, and F. W. Anderson has recognised three *Cypridea* zones. Field mapping indicates that certain stratigraphical names, including "Fairlight Clays", should be abandoned.

Detailed description of Wealden sections occupies fifty-four pages. This contrasts with the brevity of the chapters on superficial structures and on economic geology, which together occupy seventeen pages. For the section on superficial structures a sketch map to illustrate areas prone to surface movement (landslipping, cambering and valley bulging) would have been useful. The chapter on economic geology consists of brief accounts of past and present working for material such as Wealden iron, brick-clay, cement and lime, building stones and oil and gas.

As such it is an interesting historical survey but of limited present day significance. Sand and gravel, for example, are discussed in three short paragraphs, despite the extensive areas of outcrop of sandy formations. Although "no large commercial organisations are utilising this source of sand at present, probably because the sand is too fine for most purposes", an assessment of possible industrial uses, or even of reserves, would be invaluable.

In summary, the authors have produced a good and attractive memoir within the limitations imposed by the standard framework of the sheet memoir series. One is left wondering for whom the Institute of Geological Sciences produces these publications, which have evolved so little in style and content. With the increased emphasis on mineral assessment and other aspects of economic geology within the institute, it could be useful to expand the economic side of the memoirs, possibly at the expense of detailing innumerable local sections.

P. F. RAWSON

Adventurous Physics

Forces and Particles: An Outline of the Principles of Classical Physics. By A. B. Pippard. Pp. xii+321. (Macmillan: Basingstoke and London, November 1972.) £5.75.

PROFESSOR PIPPARD explains in the preface that this book is an expanded version of his lectures to the "fast" stream of first year physics students in Cambridge. He refers to them as "those who wanted the more searching treatment", but certainly in my time the two courses which ran in parallel were referred to as "fast" and "slow". These are relative terms; to quote again from the author's preface, "Recognizing the impossibility of pleasing everyone, I have now addressed myself, as I did when giving the course, to the more adventurous who are prepared to work out for themselves congenial patterns of thought that will make sense of what can so easily remain a disorderly assembly of observations and ideas". This is not, therefore, a book for the average student, and it will not make a fortune for its publisher or its author. It is, however, distinctly possible that it will be an influential book, and it should be read by every physics lecturer, for we are in some danger of forgetting that these topics can be treated with this degree of elegance, economy and clarity.

The coverage is not as wide as the subtitle might suggest; it is restricted to dynamics and electricity and magnetism with brief incursions into relativity and introductory quantum mechanics. A few selected experiments are described in some detail. The order in which

topics are treated is on the whole not unconventional. I found only one point at which I felt the book departed from the high standard of exposition and logical treatment which characterizes it; this is on page 194 where *D* makes a sudden appearance in circumstances where it might be confused with a depolarizing coefficient *D*, to disappear incidentally after the following page and find no mention in the chapter entitled "Maxwell's displacement hypothesis". There are some characteristically Pippardian asides on topics such as numerology. God, like *D*, is briefly admitted to a possible role (page 130) but is not in the index.

Altogether this is an excellent book in the best traditions of the Cavendish.

W. COCHRAN

A Modern Icarus

Flight and Nature. By M. W. Saunders. Second edition. Pp. vi+126. (M. W. Saunders: Chaldon, Surrey, 1972.) £4.80.

IN a book largely concerned with a theoretical synthesis of man-powered ornithopters, it is strange to find an introduction reiterating all the usual statements on man's ecological responsibilities, however valid such statements may be. The author seems to feel that the study of natural flight is a duty, though he has apparently made no investigations himself. As he puts it: "The main reason for studying ornithoptics and aero-ecology concerns the long term maintenance of our heritage. A broad and continuous investigation would seem appropriate". He also suggests that there is a "likelihood of beneficial offshoots concerning such matters as birdstrikes, aerodynamics, air traffic, etc.". It seems that the author believes that little is known about the flight of birds. His list of references makes no mention of some of the most valuable recent contributions.

The theoretical study is straightforward, though perhaps oversimplified in parts; but where he considers aspects of practical design the proposals are very questionable. There seems to be no realisation of the stresses on the structure, hinge joints, and so on, due to inertial loads. Frictional losses within the mechanism are dismissed; power transfer is supposed to be carried out by wires which the author says must be designed "to have a very low sliding friction. For example, they may slide in guide tubes and be supported: by a fluid medium, by rollers, or by coaxial springs". His proposals for instrumentation are even more obscure: "Information on the craft's performance could be conveyed to the aviator by means of earphones, such information could be verbal, or in the form of sounds related directly with performance.

Direct acoustic connection to the wing surface could be used if preferred".

There is reason for believing that any attempt to achieve man-powered flight by flapping wings must fail. The mechanical limitations imposed by the physiology of the living animal, such as blood circulation and growth, permit locomotion only by the use of oscillatory systems with their inherent problems of inertial forces. Such forces may well set a limit to the size of birds. Man-made devices using rotary motion are not limited by such alternating stresses.

It is true that, as the author says, there is much yet to be learnt about natural flight, but it will not be learnt by this approach. R. H. J. BROWN

Thinking Computers

Problems of Heuristics. Edited by V. N. Pushkin. Translated from Russian. Pp. vi+202. (Israel Program for Scientific Information: Jerusalem; John Wiley: Chichester, March 1973.) £8.45.

THIS book is a translation—a very competent one—of a collection of papers published by the Institute of Psychology of the Academy of Pedagogical Sciences of the USSR in 1969. It gives one a fascinating glimpse into the current thinking of Russian psychologists about human thought and the problems of describing it in programmatic terms. Apparently there is as much lively discussion in the USSR as in the West of the possibilities and limitations of computer models of psychological processes; whatever the future of artificial intelligence and heuristic programming, it is good to see the issues discussed in such a thoroughgoing fashion.

The authors represent a wide variety of disciplines, including psychology, logic, pure mathematics and engineering. Their interests are reflected in the structure of the book, which is divided into four parts. Parts 1 and 2, entitled "Theoretical Problems and Heuristics" and "Experimental Studies and Heuristic Processes" respectively, are intellectually dominated by the editor's own contributions. He sees clearly the need to study human performance in order to construct effective algorithms for automatic process control and problem solving, and draws attention to the serious deficiencies of "stimulus-response" automata as models of human problem solving. What we need, he suggests, is a theory of "heuristic automata" which could model the elements of a given problem, construct systems from these elements and use the systems for solving the problem. Unfortunately there is no general theory of such automata at the present time.

The other two parts of the book are entitled "Programming and Components of Human Thought" and "Investigation of Reflexive Processes". The latter is the

shortest section and the most idiosyncratic; it deals with "devices which optimise their performance in response to human counteraction", and it is not clear that they have very much to tell us about human thought. But the main body of the book, represented by parts 2 and 3, is a mine of case studies of human beings attempting to solve simple (and not so simple) intellectual problems. There is a thoroughly professional chapter on the perception and recollection of board positions in chess, this study being backed up by observing the eye movements which accompany the examination of a board position in the process of attempting to memorise it or to solve a chess problem. There is a comparison between the performance of humans and computers in solving various kinds of rearrangement puzzles and a very long chapter on the famous problem of the fly which travels to and fro between two cyclists riding towards each other—though perhaps it generates more amusement than psychological insight. In part 3 the most interesting paper is one on the automatic design of pipelines, an exercise in cooperation between human designers and computing machines. It seems to be inspired by a good sense of the proper place for computers in human society.

H. C. LONGUET-HIGGINS

Medical Thoughts

Medicine and Society. By H. Miller. Pp. 87. (Oxford University: London, August 1973.) £2.25 library edition; 80p paper.

THIS is an excellent book that ought to be read by anyone with the slightest interest in medicine and medical services. In eighty pages Dr Miller gallops through (*inter alia*) drug development, operation of the National Health Service, staffing of hospitals, general practice, population screening, education, dentistry, genetics, abortion, euthanasia, transplants, contraception and the medical needs of the third world. He says something interesting and provocative about them all, and doesn't waste a word.

The Medical Research Council and molecular biology are rapidly dispatched in "it is still difficult to escape the impression that the collection of Nobel prizes and the headlong pursuit of the almost metaphysical harvest of molecular biology has taken precedence over the exploitation of science in the service of medicine and man". He clearly dislikes any thought that euthanasia might be available through the medical profession: "It might be more appropriate for an enthusiastic prelate to conduct the patient to the Elysian fields".

This is just the book for a ninety-minute train journey.

DAVID DAVIES

Rationalizing Water

Scientific Allocation of Water Resources: Water Resources Development and Utilization—A Rational Approach. By Nathan Buras. Pp. 208. (American Elsevier: New York; Elsevier: Amsterdam, 1972.) Dfl.46; \$14.50.

THE last decade has seen both a much greater awareness of the many severe and growing complications (technical, legal and social) in water resources studies and the development of powerful systems engineering techniques. The techniques, however, cannot yet cope fully with all the complexities of the overall water resources problem. Either the problem has to be simplified in certain aspects or the techniques are applied to particular components of the overall problem.

In the latter category, Buras has made notable contributions to the development and application of systems techniques in water resources engineering. His book shows succinctly (in 200 pages) his authoritative grasp of the subject and is to be welcomed for bringing together material dispersed over a plethora of journals and proceedings.

The preface and introduction give respectively lucid surveys of the book itself and of the historical background of water resource development from Biblical times onwards. The overlap of water resources engineering with many related disciplines in the pure and applied sciences and in the social sciences is well summarized in the introduction.

Chapter 2 sets out the philosophy of the systems approach to water resources studies, without obscuring that philosophy with mathematics. Summaries of applications of the approach in California, the Indus Valley and Israel are presented. Wisely, Buras stresses that the application of systems engineering to water resources problems is only in its initial exploratory stage.

After distinguishing between development problems, design problems and operational problems in chapter 3, he gives a full account of those probabilistic methods applied in water resources studies, with the minimum of mathematical obscuration, in chapter 4.

The optimization techniques widely used in water resources problems, and to which Buras has himself made many contributions, are set out in chapters 5, 6 and 7. Linear programming, with specific examples to leaven the basic material, is adequately covered in chapter 5. The main value of the whole book, however, may well be the thorough treatment given to dynamic programming methods, both as a way of approach and in their application, in chapters 6 and 7. These chapters seem to me to be the outstanding feature of the book, and will provide a sound insight to this complex subject. A brief

review of simulation methods is given in chapter 8.

This book fills a gap in the texts on water resources engineering. It deserves to be studied by all graduate students in the water resources field, and should be read by many others in the associated disciplines. T. O'DONNELL

How Herbicides Work

Mode of Action of Herbicides. By Floyd M. Ashton and Alden S. Crafts. Pp. 504. (Wiley Interscience: New York and London, February 1973.) £12.50.

THIS book is a mine of information. It opens with a few general chapters on the economics of weed control and on the classification of herbicides and then proceeds to give brief comparative consideration of factors affecting uptake and translocation of herbicides, on their molecular fate in the plant and on the main kinds of biochemical responses of plants to their presence. This is followed by fifteen chapters, together occupying the greater part of the book, each dealing with a chemically defined group of related herbicides.

Each of these specialized chapters is arranged on a common plan. First, the herbicides themselves are defined and described. Next, their general macroscopic effects on plants are outlined and our knowledge of their uptake and translocation is summarized. Then follow sections on metabolic change of the herbicides in the plant, if any, and on the known biochemical responses of the plant in the period preceding death. This latter is usually the largest section in each chapter. A final section summarises present views on which of the induced biochemical abnormalities are most clearly associated with plant death. Each chapter is provided with a well chosen set of references so that any point of special interest to the reader can be easily followed up.

The modern herbicides are virtually all products of the last thirty years or so; it is really notable how much work has been done on their physiological and biochemical effects. Two main observations can be made on the results of this work. First, it is clear that very few herbicides indeed have a simply definable mode of action, at least in our present state of knowledge. Perhaps the best example of a simple mode of action is that of the nitrophenols; these compounds have been well known to biochemists for a long time as uncoupling agents and it seems that it is indeed this activity which is mainly responsible for their toxicity to plants. At the other extreme, the phenoxy-acetic acid hormone-type weed killers induce an enormous number of unrelated biochemical responses and we are still, after thirty years research, far

from being able to account for their important herbicidal activity.

The second general observation is that it is striking that few, if any, herbicides show a true specificity of action. Selectivity, when it occurs, is often based on crude mechanisms based on differential wettability of leaf surfaces, ease of uptake and translocation or access to root systems. Sometimes a more sophisticated mechanism is involved where selectivity is based on differential capacity to degrade the herbicide to less toxic derivatives. Specificity based on action at a specific site is not known, contrasting in this respect with some of the more selective antibacterial antibiotics.

This is a book which will interest not only the specialist working on herbicides but also many with a more general interest in the action of toxicants on living cells. It is a book that many would like to have as a reference book on their own shelves, but the price is scarcely encouraging.

P. W. BRIAN

Coastal Problems

The Water's Edge: Critical Problems of the Coastal Zone. Edited by B. H. Ketchum. (Coastal Zone Workshop held 22 May–3 June, 1972, Woods Hole, Massachusetts.) (MIT: Cambridge, Massachusetts and London, 1972.) £1.80; \$3.95.

A RECENT review of scientific, economic, social and political problems of coastal zones has been published from MIT—a result of a “workshop” jointly sponsored in 1972 by the US Institute of Ecology and the Woods Hole Oceanographic Institution and supported by NSF (RANN Program) and the Rockefeller Foundation.

As Hollings¹ has observed of another, similar exercise “the real success of the workshop rests as much with its style of operation as with its substance” but of its method of operation the book gives little clue. Chapters have been prepared by small, presumably editorial, groups, with “contributions from . . .”. This technique provides a pleasant uniformity of style, but one wonders how much dissension, argument and genuine difference of view has been incorporated.

The report considers, in turn, diverse aspects of coastal zone problems—those arising from cropping biological resources; from non-renewable resources of oil, gas, minerals, gravel; those posed by growing demands for recreation and amenity; those changes brought by urbanization, industrial development, navigation and waste disposal schemes.

It effectively distinguishes between exclusive uses of the resource (such as aquaculture, or nature reserves), multiple uses (such as water for industry, waste disposal) and displaceable uses (that is, those such as power stations which could

be located elsewhere), and sagely observes that the present sad state of many coastal sites is a result of their non-management as much as mismanagement.

From the British standpoint both comparable and contrasting views are indicated. First, the coastal zone is “a band of dry land and adjacent ocean space”—in effect extending over coastal plains, or even whole watersheds, to the edge of the continental shelf and beyond. The United Kingdom concept of coastal areas, by contrast, appears parochial, if more closely focused. Perhaps inevitably, the same conflicts of use emerge in both the USA and UK, and there is the same divisive confusion of land use planning, management and legislative authorities. It becomes a truism that integrated and well-managed use of these areas demands a closely knit, well co-ordinated, overall agency, vested with suitable powers to make decisions effective, and with the wisdom of a Solomon, to boot.

In a series of far-reaching recommendations the MIT reports calls for: a national coastal zone policy to determine priorities for future uses and to maintain natural ecosystems; a coastal zone task force to develop management schemes and model guidelines for regional and local plans; for revision of legal institutions and procedures to make management more effective; for establishment of coastal zone centres to carry out and coordinate scientific, social and legal research programmes; for a national system of coastal area preserves with closely restricted access, to protect basic genetic stocks.

The problems exposed are those we face nearer home, the ideal solutions much the same as those we would choose: will the Americans be more successful than we in devising a fair, rational and effective scheme of regional control? The cards seem stacked against them: to quote: “67% of California's estuarine habitats lost in twenty years”; “of recreational shoreline, 92% was privately owned”; “booming coastal industry” which includes increased dredging and filling, increased developments in underwater mining and oil extraction.

UK problems of the coastal zone have been considered, in part, by the Royal Commission². The new Regional Water Authorities will be able to tackle some effects of our present disorganization, but will be short on skills and resources in the aquatic field, at least initially, as well as restricted in the scope of their interest. Surely the need is now for the UK to look at wider aspects of coastal zone management. The growing offshore extraction industries and our developing links with other riparian countries bordering coastal waters will present

many problems in the years ahead that a rational management scheme could overcome. Perhaps the Royal Commission could extend its sights beyond estuaries, to the coast and nearshore waters, to identify important issues and to achieve an overall assessment of our needs, both now and in the future.

The MIT report represents a rational and sensible approach to these problems, and no one sector has been able to press its own problems at the expense of the others. This, in essence, is the virtue of the openness and flexibility of the workshop approach. But the shining ideals and grand concepts need both the will, the machinery, and the funds to advance further. The provision of these, both in the UK and US, is where the real challenge lies.

G. HOWELLS

¹ "Resource Science: The nurture of an infant", *Bioscience*, 23, 13 (1973).

² Third Report of the Royal Commission on Environmental Pollution, Cmnd 5054 (HMSO, 1972).

Activities of Wasps

The Wasps. By H. E. Evans and M. J. W. Eberhard. Pp. vi+265. (David and Charles: Newton Abbot, July 1973.) £3.65.

THE wasps are all those sting-bearing or aculeate Hymenoptera which are not ants or bees. About half of these are wasps in the more restricted sense—species which build nests and store food in them for their young. The ability to display this degree of maternal care involves many new behavioural traits; having a nest, for example, means having a sense of locality and a good memory and these in turn make such wasps a fascinating study because of the contrasts between some quasi-human behaviour and much rigid instinctive activity.

Dr Evans is an authority on the diggerwasps, a large group of solitary species on which he has published much original work. They show all stages from the simplest in which the wasp stores paralysed prey and lays an egg on it but then seals the cell to such species as *Amonophila pubescens* which brings her larva food as required, over several days, and is able to run two nests simultaneously, in different stages of development and requiring different treatment.

The other author, Dr Eberhard, has made brilliant studies of *Polistes* wasps. The colonies of these insects are very convenient for observation, the single combs being usually completely exposed, often hanging from the eaves of houses and the colonies being mostly small so that the individuals can all be marked with paint and their activities separately recorded. Species are found in both temperate and tropical climates and the

resulting differences in behaviour enable us to see better what elements are fundamental and what are adaptations to local conditions. Thus in temperate climates only the fertilized females survive the winter while the males and workers die. To survive, the female has to be rather larger and to lay down a fat reserve of a rather different form. In the spring she can found a new colony unaided until her first daughters emerge. In the tropics, colonies would be perennial except that, when the dominant egg-laying female begins to fail, the colony disperses, usually founding new colonies with swarms.

It is not yet certain that in such species there is any distinction between egg-laying female and workers except a behavioural one, the egg-laying female being able to prevent the others from laying, so that they become workers by default. In such wasps there is still need for research to settle whether there is a caste of real workers which could not alone found new colonies. The more advanced social wasps which are also described often make much larger nests with a beautiful and elaborate structure, inhabited by thousands of wasps which in some genera may include a large number of egg-layers. In these wasps the egg-layers are sometimes quite distinct and easily distinguishable from the workers to the naked eye, as in the British social wasps. All these and many other topics are ably and lucidly discussed in this valuable introduction.

O. W. RICHARDS

Motile Models

Motile Muscle and Cell Models. By N. I. Arronet. Translated from the Russian by Basil Haigh. (Studies in Soviet Science.) Pp. ix+192. (Consultants Bureau: New York and London, 1973.) \$29.

THE title of this unusual book may mislead the prospective reader. What constitutes a model of a muscle or motile cell? By a model one often means a physical object or a mathematical theory which reproduces the important properties of a more complex system. But in the present context, model is used in the sense of a copy of the original. In 1946 Varga in Szent-György's laboratory introduced the glycerol-extracted muscle fibre as a model of a living muscle. Treatment with glycerol destroys the cell membrane or greatly increases its permeability and removes metabolites and soluble enzymes. What remains is a working model of the muscle which can be manipulated by the experimentalist since it is permeable to substrates and inhibitors of the contractile proteins. Whether the properties of the model reproduce those of the living muscle must be determined by experiment.

Because the contractile proteins are relatively insoluble the model has proved to be a remarkable likeness of the original. Nevertheless there are differences, particularly in regard to inhibition of ATPase activity in the relaxed state.

The preparation of a glycerinated model is a simple technique, and has consequently been applied to a variety of presumed motile systems, including cilia, sperm, dividing cells, amoebae (and other organisms which exhibit protoplasmic streaming), myonemes of *Vorticella* mitochondria, and chloroplasts. The criterion for a successful model is simply the production of some form of movement on addition of ATP or other substrates. If the original is a highly ordered structure whose mechanical properties have been determined, one can establish that the model is a reasonable copy of the original. The technique has been successfully applied to cilia and flagella particularly by Gibbons who has obtained wave propagation and swimming movements with glycerinated sperm. For other systems it is difficult to decide whether the essential properties have been preserved and whether the response observed bears any relation to the normal function.

Arronet's book is a survey of studies on glycerinated models of all kinds. One use of models is for teaching purposes and perhaps the most interesting feature of this book is a collection of recipes for making models. Practical details are stressed and many of the methods seem to have been tested by the author.

The remainder of the book is a review of the literature on model studies. The presentation is more in the form of a recitation than a critical discussion. As long as the model is prepared from a muscle or cilium, one is on relatively firm ground, but for other systems extreme caution is required. Discussion of work on mitochondria and chloroplasts will serve as an example. Addition of ATP to these organelles, whether glycerinated or not, can lead to changes in shape and volume. The presence of a myosin-like contractile protein has been claimed and denied by a number of workers and at present it seems likely that the volume changes can be explained by ion movements. Yet Arronet concludes on the basis of ATP response of glycerinated mitochondria and chloroplasts that they "share a common mechanism of contraction with muscles, enzymatic interaction between the contractile protein and ATP".

Glycerinated models have been and will continue to be an important method in the study of motile systems. This book provides a useful collection of such studies and will serve as a valuable reference.

E. W. TAYLOR

CORRESPONDENCE

Smoking and Pregnancy

SIR,—Some of us decline to accept Dr Goldstein's own evaluation of his criticisms¹⁻³. He states³, as an "undisputed" fact, that "... the distribution of birthweights for babies of smokers is the same as that for non-smokers except that it is shifted downwards by about 170 g". (This is known as the 'displacement hypothesis'.) It follows that the mean birthweight of infants born to smoking mothers should also be "about 170 g" less than that for babies born to non-smoking mothers. In Yerushalmy's study⁴, the observed differences in the mean birthweights were 209.1 g for "whites" and 204.8 g for "blacks". By suitably stretching our imaginations we can, perhaps, regard these as being "about 170 g". However, Yerushalmy⁵ also tested the hypothesis that the distributions of low birthweights, allowing a displacement of 200 g between the smoking and non-smoking series, are the same. He found that the distributions differ significantly ($0.005 < P < 0.01$). The 'displacement hypothesis' is therefore rendered improbable by Yerushalmy's⁵ findings.

So much for "undisputed" facts. Apart from raising doubts about Goldstein's most confident assertions, they do not affect Yerushalmy's arguments. To quote Yerushalmy⁵, "... the major conclusions in the paper (ref. 4) do not rest on the results of the displacement hypothesis". In particular, contrasts between the findings for infants of smoking mothers and smoking fathers cannot easily be reconciled to a cause-effect hypothesis⁴. Although smoking mothers, and especially smoking fathers, had a higher incidence of low-birthweight babies than corresponding non-smokers, prognosis (death and congenital anomalies) for the low-birthweight babies of smoking fathers was much worse than for low-birthweight babies of smoking mothers⁴. By far the best prognosis was given for infants of matings in which the mother smoked and the father did not. This finding applied both to "whites" and "blacks". Furthermore, the general unsoundness of arguing from simple association to cause was emphasised by the large biological and mode-of-life differences demonstrated between smoking and non-smoking gravidas.

Now to answer Goldstein's second point³. Yerushalmy⁶ found that women who began to smoke after the birth of their baby had a much higher incidence ($\times 1.8$) of low-birthweight babies than those who did not take up smoking. His investigation was confined to all

births that occurred in women aged twenty-five or less, and in women who started to smoke in this age-range. Goldstein³ argues that the observed difference in the incidence of low-birthweight babies arises because mothers who started smoking before twenty-five years of age would have had their babies at an earlier age than those who did not take up smoking: younger mothers, he claims, have lighter babies. Yerushalmy⁷ has previously shown that his findings do not support this objection: "... within the limited range of ages under twenty-five years the variation in the proportion of low-birthweight infants is very small and could not account for the large differences found." He supported his argument with a table showing the mothers' age, total live births, and the numbers and incidence of low-birthweight infants⁷. We can split the age-range conveniently into two groups to give equal numbers (164) of low-birthweight infants: for mothers in the age-range fifteen to twenty-one years, the incidence of low-birthweight babies was 6.2% (164/2639); and for those in the age-range twenty-two to twenty-five it was 5.6% (164/2906). The small difference does not approach significance (χ^2 with Yates correction = 0.71; $0.3 < P < 0.5$). By contrast, the differences in the incidence of low-birthweight babies born to mothers in different smoking categories were large and significant. For "white" non-smokers, the incidence was 5.3%, but for "white" mothers who took up smoking after the birth of their baby it was 9.5% ($P < 0.01$)⁶. The corresponding incidence⁶ for mothers who smoked during pregnancy was 8.9%; and for those who gave up smoking after the birth of their baby it was 6.0%, similar to that for non-smokers. Trends for "blacks" resembled those for "whites", but because of smaller numbers, the only statistically significant difference was between those mothers who gave up smoking after the birth of their baby—with an incidence of low-birthweight babies of 13.4%—and those who smoked both during pregnancy and afterwards—with an incidence of 19.9% ($P < 0.02$). This collective evidence⁶ therefore fails to corroborate the causal hypothesis. Each of its features is remarkably consistent with the view that the smoker, rather than the smoking, is responsible for the high incidence of low-birthweight infants^{6,7}.

Finally, I return to the questions raised in your editorial of September 14. A news story based on an accurate

report of an article by Goldstein opened with the statement that cigarette smoking during pregnancy "caused the deaths of 1,500 babies in Britain last year". Under pressure from your leading article, Goldstein conceded in these columns, on October 5, "... the scientific evidence for a causal relationship may not be very conclusive". These dual standards raise important issues.

The public should be able to trust definitive statements made by scientists in their own field of expertise. If subsequently this trust proves to be unfounded, the credibility of scientists will be undermined, not only to their detriment, but to that of society at large.

Yours faithfully,

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¹ Goldstein, H., *Am. J. Epidemiol.*, **95**, 1 (1972).

² Goldstein, H., *Am. J. Obstet. Gynec.*, **114**, 570 (1972).

³ Goldstein, H., *Nature*, **245**, 467 (1973).

⁴ Yerushalmy, J., *Am. J. Epidemiol.*, **93**, 443 (1971).

⁵ Yerushalmy, J., *Am. J. Epidemiol.*, **95**, 2 (1972).

⁶ Yerushalmy, J., *Am. J. Obstet. Gynec.*, **112**, 277 (1972).

⁷ Yerushalmy, J., *Am. J. Obstet. Gynec.*, **114**, 571 (1972).

SIR,—We join Professor Burch¹ in welcoming your leading editorial² on "smoking, pregnancy and publicity", and for noting that the elementary fallacy of inferring causality from statistical association is present in some reports that have received publicity.

In response to your editorial, Goldstein³ cites a *British Medical Journal* editorial⁴ that he states "suggests" validity of the smoking-causality hypothesis as an explanation for the statistical association between smoking behaviour of women and birth weights of their children. An alternative hypothesis, set forth by Professor Yerushalmy, is that the smoker rather than smoking influences birth weight⁵. Another expression of this hypothesis is that "smoking behaviour of women and birth weights of their children are influenced by a common cause ... the individual genotype or constitution"⁶. The *British Medical Journal* editorial⁴ does not merely suggest, but asserts that "no reasonable doubt now remains that smoking in pregnancy has adverse effects on the developing foetus". As we have noted⁶, the editorial overlooked

several reports that disagree, especially those of Yerushalmy.

Ross and colleagues⁷, and Goldstein³, deny having inferred causality from correlation. To the causal quotation cited in the *Nature* editorial² may be added their own assertion⁸, based on statistics, that "cigarette smoking during pregnancy increased the foetal plus neonatal mortality rate by 28% and reduced birth weight by 170 g. . . ." They also assert⁷ that the constitutional hypothesis as evaluated by Yerushalmy⁵ "suffers from severe methodological shortcomings, making it clearly untenable", citing a critique by Goldstein⁹. However, they neglect to mention Yerushalmy's rejoinder¹⁰ which, in effect, demolished this critique⁹.

Goldstein expressed his opinion that it would be "unethical" not to advise and encourage "pregnant women, especially 'high risk' ones, to stop smoking"³. In response to similar statements by Goldstein⁹, Yerushalmy¹⁰ commented, following a data analysis, that it would be unfortunate "to recommend a course of action based on conjectures, subjective notions, and easy explanations not supported by available data." The smoking-causality hypothesis to explain the association between smoking by women and birth weights of their children is incompatible with observed data^{5,6}. Such incompatibility, if confirmed independently, requires rejection of this hypothesis.

A problem arises since human smoking behavioural subgroups, for example, smokers, non-smokers and ex-smokers, are self-selected rather than randomly selected, and thus are biased samples. This introduces special problems for statistical testing of hypotheses^{5,6}. It is quite possible that for a subgroup of all smokers, tobacco use may be symptomatic of underlying

needs, perhaps arising in part from variations in biogenic amine physiology, that tend to be alleviated by nicotine^{6,11}. Cigarette smokers tend to adjust their smoking behaviours according to the nicotine content of cigarettes¹². Accordingly, ethological homeostatic mechanisms may be involved^{6,13}.

Goldstein cites animal studies in support of his position³. In addition to the usual caveat concerning inferences from experimental animal studies to man, based on interspecific physiological differences, there is a further problem. If habituated human smokers are on the average physiologically different from non-smokers, this difference may not be reflected in the physiology of experimental animals used in tests. Moreover, biogenic amine release in non-deficient test animals above homeostatic optimal ranges may be harmful¹⁴. The fallacy of typology¹⁵ may well be involved.

Consider the following observations: the negative association of carboxyhaemoglobin (COHb) levels of women with birthweights of their children¹⁶; the low COHb levels of some smokers^{16,17}, and the limited data for angina patients showing that high COHb levels of smokers are sustained during a non-smoking period¹⁸. These are compatible with a constitutional rather than a smoking-causality hypothesis. Such observations suggest caution regarding rejection of constitutional hypotheses and allegations that smoking-causality hypotheses concerning birth weight are in fact true. In this complex problem, available objective evidence for testing alternative hypotheses must be considered fairly, without overlooking critical reports that are detrimental to the smoking-causality hypothesis⁴, or to any other hypothesis. It is to be hoped that in any scientific problem all avail-

able objective evidence may be utilised, thus minimising the need for subjective judgment and opinion.

Yours faithfully,

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